

PROCEEDINGS
OF
PAKISTAN CONGRESS OF ZOOLOGY

Volume 38, 2018

*All research articles in this Proceedings were refereed
by experts in respective disciplines*



THIRTY EIGHTH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

**DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF THE PUNJAB, LAHORE**

FEBRUARY 27 – MARCH 1, 2018

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PROCEEDINGS OF THE CONGRESS

Editor

Prof. Dr. A.R. Shakoori

Composed & Designed by: Fakhar Mahmood Shahid

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ACKNOWLEDGMENTS

Department of Zoology, University of the Punjab, Lahore hosted the 38th Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, University of the Punjab, Lahore, faculty members and students of the Department of Zoology for extending warm hospitality.

Grants were received from Higher Education Commission, Islamabad, Pakistan Academy of Sciences, Islamabad and Pakistan Science Foundation, Islamabad.

38th PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

UNIVERSITY OF THE PUNJAB, LAHORE
FEBRUARY 27 – March 1, 2018

PROGRAMME

TUESDAY, FEBRUARY 27, 2018

08:30 AM	REGISTRATION
10:00 AM	Inauguration: Recitation from the Holy Quran
10:05 AM	Welcome Address
10:15 AM	Address by the President, Zoological Society of Pakistan
10:25 AM	Distribution of Medals and Awards
10:45 AM	Address by the Chief Guest
11:15 AM	Vote of Thanks
11:25 AM	Refreshment

HALL – 1

JOINT SESSION I: (Plenary Lectures)

Chairman: Prof. Dr. A.R. Shakoori

Co-Chairman: Prof. Dr. Muhammad Ali

- Speakers: 1. **Prof. Dr. Wei Miao,**
*Institute of Hydrobiology, Chinese Academy of Sciences,
Wuhan, People's Republic of China.*
The Adaptive Evolution of Ciliated Protozoan
2. **Prof. Dr. Mohammad Perwaiz Iqbal,**
*Department of Biological and Biomedical Sciences, The Aga
Khan University, Karachi.*
**Hypovitaminosis D and Coronary Artery Disease in
Pakistan**
3. **Prof. Dr. Telat Yanik,**
Department in Fisheries, Ataturk University, Erzurum, Turkey.
Future Trends for Sustainable Aquaculture in Turkey

01:00 PM **Lunch and Prayer Break (Zuhar)**

HALL – 1**SECTION I: CELL AND MOLECULAR BIOLOGY, GENETICS****SESSION 1**

	Chairperson:	Prof. Dr. Farah Rauf Shakoori
	Co-Chairperson:	Dr. Raazia Tasadduq
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 2

	Chairperson:	Dr. Abdul Rehman
	Co-Chairperson:	Dr. Soumble Zulifiqar
05:00 PM	Paper reading	
06:25 PM	Prayer Break (Maghrib)	

SESSION 3

	Chairperson:	Dr. Nadeem Shaikh
	Co-Chairperson:	Dr. Saba Irshad
06:45 PM	Paper reading	
08:30 PM	Dinner	

HALL – 5**SECTION II: HUMAN AND ANIMAL DISEASES****SESSION 1**

	Chairperson:	Dr. Bushra Muneer
	Co-Chairperson:	Dr. Saba Shameem
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 2

Chairperson: Dr. Irfan Zia Qureshi
Co-chairperson: Dr. Abdul Rauf Janjua
05:00 PM Paper reading
06:25 PM Prayer Break (Maghrib)

SESSION 3

Chairperson: Prof. Dr. Aneela Zamir Durrani
Co-Chairperson: Dr. Fariha Arooj
06:45 PM Paper reading
08:30 PM Dinner

HALL – 2

SECTION IV: PEST AND PEST CONTROL

SESSION 1

Chairperson: Dr. Mushtaq A Saleem
Co-Chairperson: Dr. Munawar Saleem Ahmad
02:00 PM Paper reading
04:30 PM Tea Break and Prayer Break (Asar)

SESSION 2

Chairperson: Dr. Inamullah Khan
Co-Chairperson: Dr. Imran Khatri
05:00 PM Paper reading
06:25 PM Prayer Break (Maghrib)

SESSION 3

Chairperson: Prof. Dr. Nasreen Memon
Co-Chairperson: Dr. Noor Muhammad
06:45 PM Paper reading
08:30 PM Dinner

HALL – 3

SECTION V: FISHERIES, FRESHWATER BIOLOGY

SESSION 1

	Chairperson:	Prof. Dr. Naeem Tariq Narejo
	Co-Chairperson:	Dr. Shafique Ahmad
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 2

	Chairperson:	Dr. Muge Hekimoglu
	Co-Chairperson:	Dr. Zafar Iqbal
05:00 PM	Paper reading	
06:30 PM	Tea Break and Prayer Break (Maghrib)	

SESSION 3

	Chairperson:	Prof. Dr. Muhammad Shoaib
	Co-Chairperson:	Dr. Abdul Majid Khan
06:45 PM	Paper reading	
08:30 PM	Dinner	

HALL – 4

SECTION VI: PARASITOLOGY

SESSION 1

	Chairperson:	Prof. Dr. Abdullah Arijo
	Co-Chairperson:	Dr. Noor-un-Nisa
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 2

Chairperson: Dr. Ali Murtaza Dharejo
 Co-Chairperson: Dr. Saima Naz
 05:00 PM Paper reading
 06:30 PM Tea Break and Prayer Break (Maghrib)

SESSION 3

Chairperson: Prof. Dr. Asmatullah Kakar
 Co-Chairperson: Dr. Muhamamd Mazhar Ayaz
 06:45 PM Paper reading
 08:30 PM Dinner

DAY TWO: WEDNESDAY, FEBRUARY 28, 2018

JOINT SESSION II: (Plenary Lectures)

9:00 AM

Chairman: Prof. Dr. Javed Iqbal Qazi
Co-Chairman: Prof. Dr. Wei Miao

- Speakers: 1. **Prof. Dr. Christine Blattner,**
Institute of Toxicology & Genetics, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany
The Good and Bad of p53
2. **Dr. Muhammad Khan,**
College of Basic Medical Sciences, Dalian Medical University, Dalian, China
What Made Alantolactone a Lead for Anticancer Drug Development?
3. **Dr. Muge Hekimoglu,**
Aquaculture Department, Ege University, Bornova, Izmir, Turkey
Development of Aquaculture and Aquarium Sector in Turkey

HALL – 1**SECTION I: CELL AND MOLECULAR BIOLOGY, GENETICS****SESSION 4**

	Chairperson:	Prof. Dr. Christine Blattner
	Co-Chairperson:	Dr. Dil Ara Abbas Bukhari
10:45 AM	Paper reading	
11:45 PM	Tea Break	

HALL – 1**SECTION VII: PHYSIOLOGY****SESSION 1**

	Chairperson:	Prof. Dr. Syed Shahid Ali
	Co-Chairperson:	Dr. Bibi Nazia Murtaza
02:00 AM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 2

	Chairperson:	Dr. Nuzhat Shafi
	Co-Chairperson:	Dr. Chaman Ara
05:00 PM	Paper reading	
06:30 PM	Tea Break and Prayer Break (Maghrib)	

SESSION 3

	Chairperson:	Dr. Nabila Roohi
	Co-Chairperson:	Dr. Faiza Jabeen
06:45 PM	Paper reading	
08:30 PM	Dinner	

HALL – 2

SECTION IV: PEST AND PEST CONTROL

SESSION 4

	Chairperson:	Dr. Alam Zeb
	Co-Chairperson:	Dr. Faheem Khan
10:45 AM	Paper reading	
11:45 AM	Tea Break	

SESSION 5

	Chairperson:	Dr. Abdul Aziz Khan
	Co-Chairperson:	Dr. Nasira Khatoon
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 3

	Chairperson:	Prof. Dr. Muhammad Saeed Wagan
	Co-Chairperson:	Dr. Tanzeela Riaz
05:00 PM	Paper reading	
06:30 PM	Tea Break and Prayer Break (Maghrib)	

HALL – 2

SECTION VIII: ENTOMOLOGY

SESSION 2

	Chairperson:	Dr. Nusrat Jahan
	Co-Chairperson:	Dr. Aysha Ihtisham
06:45 PM	Paper reading	
08:30 PM	Dinner	

SESSION 2

Chairperson: Dr. Nusrat Jahan
 Co-Chairperson: Dr. Aysha Ihtisham
 06:45 PM Paper reading

HALL – 3**SECTION IX: WILDLIFE, BIODIVERSITY****SESSION 1**

Chairperson: Dr. Zulfiqar Ali
 Co-Chairperson: Dr. Muhammad Rais
 10:45 AM Paper reading
 11:45 AM Tea Break

SESSION 2

Chairperson: Dr. Abdul Aleem Ch.
 Co-Chairperson: Dr. Maqsood Anwar
 02:00 PM Paper reading
 04:30 PM Tea Break and Prayer Break (Asar)

SESSION 3

Chairperson: Prof. Dr. M. Siddique Awan
 Co-Chairperson: Prof. Dr. Ali Muhammad Yousafzai
 05:00 PM Paper reading
 06:30 PM Tea Break and Prayer Break (Maghrib)

SESSION 4

Chairperson: Dr. Bushra Allah Rakha
 Co-Chairperson: Dr. Ansar Ahmed Abbasi
 06:45 PM Paper reading
 08:30 Dinner

HALL – 4**SECTION X: ECOLOGY & ENVIRONMENTAL POLLUTION****SESSION 1**

	Chairperson:	Dr. Riffat Sultana
	Co-Chairperson:	Dr. Abdul Qadir
10:45 AM	Paper reading	
11:45 AM	Tea Break	

HALL – 4**SECTION XI: MARINE BIOLOGY****SESSION 1**

	Chairperson:	Dr. Mukhtiar Ahmed Mahar
	Co-Chairperson:	Dr. Noor-us-Sehar
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 2

	Chairperson:	Dr. Quddusi B. Kazmi
	Co-Chairperson:	Dr. Azra Bano
05:00 PM	Paper reading	
06:30 PM	Tea Break and Prayer Break (Maghrib)	

HALL – 4**SECTION XII: PALAEONTOLOGY****SESSION 1**

	Chairperson:	Prof. Dr. Muhammad Akhtar
	Co-Chairperson:	Dr. M. Akbar Khan
06:45 PM	Paper reading	
08:30 PM	Dinner	

HALL – 5**SECTION II: HUMAN AND ANIMAL DISEASES****SESSION 4**

	Chairperson:	Dr. Muhammd Khan
	Co-Chairperson:	Dr. Farzana Rashid
10:45 AM	Paper reading	
11:45 AM	Break	

SESSION 5

	Chairperson:	Dr. Najma Shaheen
	Co-Chairperson:	Dr. Tariq Zahid
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

SESSION 6

	Chairperson:	Dr. Shahid Nadeem
	Co-Chairperson:	Dr. Hafiz Abdullah Shakir
05:00 PM	Paper reading	
06:30 PM	Tea Break and Prayer Break (Maghrib)	

SESSION 7

	Chairperson:	Dr. Asmatullah
	Co-Chairperson:	Dr. Muhammad Arshad
06:45 PM	Paper reading	
08:30 PM	Dinner	

DAY THREE: THURSDAY, MARCH 1, 2018

JOINT SESSION III: (Plenary Lectures)

9:00 AM

Chairman: Prof. Dr. Muhammad Naeem Khan

Co-Chairman: Prof. Dr. Telat Yanik

- Speakers: 1. **Prof. Dr. Akram Shah,**
Department of Zoology, University of the Punjab, Lahore.
Situation Analysis of Cutaneous Leishmaniasis in Kuwait Teaching Hospital, Peshawar with Special Reference to Perception of *Leishmania* exacerbating pregnancy.
2. **Dr. Riffat Sultana,**
Department of Zoology, University of Sindh, Jamshoro
Outstanding Characteristics of Some Classic Orthopteroid Species of Pakistan
3. **Prof. Dr A.R. Shakoori,**
School of Biological Sciences, University of the Punjab, Lahore
Role of Adhesion Proteins in Metastasis

HALL – 1**SECTION II: HUMAN AND ANIMAL DISEASES****SESSION 8**

	Chairperson:	Dr. Javid Iqbal Qazi
	Co-Chairperson:	Dr. Faiza Saleem
10:45 AM	Paper reading	
11:45 AM	Tea Break	

SESSION 9

	Chairperson:	Dr. Zahoor Qadir Samra
	Co-Chairperson:	Dr. Iram Liaqat
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

HALL – 2**SECTION VIII: ENTOMOLOGY****SESSION 3**

	Chairperson:	Dr. Abida Butt
	Co-Chairperson:	Dr. Hafiz Muhammad Tahir
10:45 AM	Paper reading	
11:45 AM	Tea Break	

SESSION 4

	Chairperson:	Dr. Nasreen Muzafar
	Co-Chairperson:	Dr. Shafqat Saeed
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

HALL – 3

SECTION II: HUMAN AND ANIMAL DISEASES

SESSION 10

	Chairperson:	Dr. Muhammad Ijaz
	Co-Chairperson:	Dr. Shabana Naz
10:45 AM	Paper reading	
11:45 AM	Tea Break	

SESSION 11

	Chairperson:	Prof. Dr. Muhammad Arshad
	Co-Chairperson:	Dr. Naveeda Akhtar Qureshi
02:00 PM	Paper reading	

CONCLUDING CEREMONY

02:30 PM	Recitation
02:35 PM	Congress Report by President ZSP
02:50 PM	Award Ceremony
03:15 PM	Concluding Remarks by the Chief Guest
03:25 PM	Vote of Thanks
03:30 PM	Refreshment

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 Nasreen, S.
 Sadia
 Tariq, F.
 Zahid, H.

OKARA

Wajid, M.M. (Dr.)

PESHAWAR

Ahmad, B. (Dr.)
 Huma, Z.
 Khan, I.
 Khan, Z.
 Muhammad, N.

RAWALAKOT (AJK)

Bibi, A.
 Irshad, M.
 Khan, S.M.
 Mughal, S.H.
 Muhammad, A. (Dr.)
 Mushtaq, I.
 Sheikh, A.A.
 Touseef, M.

RAWALPINDI

Abbasi, M.A.
 Afzal, A.
 Ahmed, W.
 Akhtar, F.
 Akhter, A.
 Ali, S.
 Andleeb, S.
 Anjum, M.Z. (Dr.)
 Anwar, M. (Dr.)
 Arshad, A. (Dr.)
 Arshad, J.
 Asad, M.J. (Dr.)
 Aziz, M.A (Dr.)
 Bashir, M.
 Bashir, M.
 Basit, A.
 Batool, J.
 Choudhary, M.
 Emmanuel, A.
 Farooq, M.
 Gill, S.
 Hamid, A.
 Hussain, A.
 Hussain, A.
 Kanwal, Q.
 Karim, I.
 Khan, F.
 Khan, J.
 Khan, M.
 Mahmood, T. (Dr.)
 Munawar, N.
 Naqvi, S.T.
 Nazir, S.
 Noor, M.
 Rahman, Q.
 Rais, M. (Dr.)
 Rathore, T.S.

Rifat-un-Nisa
 Saeed, M.
 Shakeel, K.
 Ullah, I.
 Waseem, M.
 Zangi, I.

SAKRAND (Sindh)
 Jalbani, S.

SARGODHA
 Afza, R.
 Majeed, M.Z. (Dr.)
 Yahya, M.

SIALKOT
 Ashraf, M.
 Dilshad, A.
 Qamer, M.
 Suneela
 Zaib-un-Nisa

TANDOJAM (Sindh)
 Arsalan, M.
 Barijo, N.M.
 Brohi, M.U.

Chandio, R.H.
 Chhajro, M.A.
 Khaskheli, J.A.
 Khatri, I. (Dr.)
 Lachhman
 Lakho, G.M.
 Mastoi, P.M.
 Mastoi, S.M.
 Nizamani, G.M.
 Rajput, Z.
 Rehman, T.
 Siddiqui, M.W.
 Soomro, A.P.
 Soomro, R.A.
 Talpur, S.A.
 Zangejo, Z.A.

THATTA (Sindh)
 Mahar, M.A. (Dr.)

UTHAL (Balochistan)
 Bano, A. (Dr.)
 Rasheed, S. (Dr.)

CITATIONS

RECIPIENT OF LIFE TIME ACHIEVEMENT AWARD 2018



Prof. Dr. Mushtaq A. Saleem

Presently Dean, Faculty of Life Sciences, University of Central Punjab,
Johar Town, Lahore

Dr. Mushtaq Saleem obtained M.Sc from Newcastle University, UK in 1980; PhD from Univ. of the Punjab, Lahore in 1990 and Postdoctoral Research from Newcastle University and Newcastle General Hospital UK in 1992-94, besides several trainings in the UK and Pakistan. He won merit scholarship throughout his academic career including Saigol Foundation, British Council and EEC Belgium etc.

During PhD research, he discovered novel field of research regarding various enzymes and macromolecules in flour beetle and likewise during Postdoctoral Research Work at Newcastle University and Newcastle General Hospital UK on “Proteases in insects, animals and human being and their role in resistance to insecticides”. His research findings were highly appreciated by different scientists such as Dr. Mantle and Dr. Wilkins in the UK. Of this work he published several research papers abroad and Pakistan including Impact Factor Journals. On this basis he won Research Productivity Allowance of Rs.1, 20,000/- from PCST, GOP and Special Science & Tech. Allowance of Rs.84,000/- from HEC.

Dr. Saleem his started career as Associate Prof. Entomology at University of Agriculture, Faisalabad in 1999. Thereafter he was selected as Prof. of Agri. (Entomology) in 2002 at BZU Multan, Guest Member of Staff at Newcastle University UK in 2005 and 2006, Professor under TTS of HEC, Professor of Biochemistry at Institute of Molecular Biology and Biotechnology, University of Lahore for about three years. He is now serving as Dean Faculty of Life Sciences University of Central Punjab, Lahore since March 2015.

So far Dr. Saleem has published 136 Research Papers in International Repute Journals in Canada, USA, UK, Poland, Bangladesh and Pakistan (out of which 94 published in Impact Factor Journals); published 10 Books/ Technical Reports; presented 156 Research Papers in various National/ International Congresses; published 173 Popular Articles: produced/ being produced 85 MS/ MPhil/ MSc (Hons)/ MSc and 22 PhD Theses; completed 14 Research Projects; delivered several radio and TV talks and arranged several Seminars/ Workshops/ Symposium for the students and farmer communities.

Dr. Saleem obtained Star Award 2002; Presidential Award: Izaaz-i-Fazeelat 2003; Best University Teacher Award 2003 and Zoologist of the year Award 2005.

Now the Zoological Society of Pakistan has recommended “Dr. Muzaffar Ahmad Life Time Achievement Award 2018” on his above-mentioned achievements.

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2018**



Mr. Mohammad Irshad

Ex-Researcher, Pakistan Agricultural Research Council, Islamabad

Mr. Mohammad Irshad had his earlier education from Islamia College Peshawar. Afterward he did his M.Sc in Zoology from Karachi University in 1963. His first practical assignment was in PCSIR Karachi where he started his research on mites and biological control. Here he described several new species of mites. Mr. M. Irshad shifted to Commonwealth Institute of Biological Control Rawalpindi in 1968. Here he contributed on natural enemies of Pentatomids, grasshopper, housefly and forest weeds. This research paved the way for future work on applied biological control of these and other insect pests. Later on he joined Pakistan Council of Agricultural Research and concentrated his research on Pest Management of pests of cotton, paddy, maize and sugarcane. His work on sugarcane was significant for biological control of Pyrilla, loss estimation of sugarcane borers and mechanical control of Gurdas borer. His research on Pyrilla was pioneer which led to future biological control of this insect pest. This research is now benefitting farmers in million and billions of Rupees. Afterwards Mr. M. Irshad started work on post-harvest of cereals. He was instrumental in estimating losses in cereals in public sector and farm level. He was part of a team which investigated fumigation techniques. He was first in Pakistan to detect resistance in storage insect pests to insecticides and fumigants. He was instrumental in establishing biological control laboratories in sugarcane mills in several parts of Pakistan. This system is continuously expanding in the country. He ventured in forgotten field of pollinators. He quantified economic benefits of pollinators of vegetables and fruits, enumerated species of bees in

Pakistan and explored preservation techniques of pollinators. He also contributed for economic cultivation of cocoa in the Philippines through control of biological control of cocoa pod bore. This initial work in 1980 paved the way for cocoa growing in southern Philippines.

He was instrumental in training of thousands of grain handlers in public sector and farmers stores in safe storage. He also trained farmers of fruits and vegetables in preserving pollinators to promote organic farming with economic benefits.

Mr. M. Irshad guided many students of higher education in research. He was examiner of several students of post graduate level. He was on editorial board of several research journals.

He wrote 2 books for higher education of Pakistan. These are on Biological control of insect pests and weeds in Pakistan and second post-harvest studies of cereals in Pakistan. These are widely referred especially the one on biological control. He has published more than 150 papers and booklets. Mr. M. Irshad contribution in different disciplines of Entomology is well recognized in Pakistan and outside Pakistan.

**RECIPIENT OF
ZOOLOGIST OF THE YEAR AWARD 2018**



Prof. Dr. Naeem Tariq Narejo

Department of Freshwater Biology & Fisheries,
University of Sindh, Jamshoro.

Prof. Dr. Naeem Tariq Narejo was born on 25th September 1963 in District Dadu, Sindh. He obtained M.Sc degree in 1987 and M.Phil in 1997 from the Department of Freshwater Biology and Fisheries, University of Sindh, Jamshoro. He has earned Ph.D degree in 2003 in Aquaculture (Fisheries) from Bangladesh Agriculture University, Mymensingh. He joined as a lecturer in the Department of Freshwater Biology and Fisheries, University of Sindh, Jamshoro in the year 1987, and was appointed as Professor in 2006. He was awarded post-doc fellowship by Network of Aquaculture Centers in Asia (NACA), Thailand in year 2005.

Up to now he has published 142 quality research papers related to basic and applied fisheries in the journal of national and international reputes. He has produced 07 Ph.D and 35 M.Phil scholars and was Principal Investigator in 06 research projects funded by various national and international organizations like HEC, PARC, PSF, WWF Pakistan and Info- Fish Malaysia. He has published two text books for graduate and undergraduate students. He has received two gold medals as outstanding research contribution in the field of Fisheries by Zoological Society of Pakistan and University of Sindh, Jamshoro in the year 2006 and 2013 respectively. On his outstanding performance he was rewarded as Best University Teacher for the year 2014 by Higher Education Commission, Islamabad. He is also life fellow of Zoological Society of Pakistan, Pakistan Fisheries Society and Aquaculture Society of Bangladesh.

*Other applicants of this award were Mr. Mudassir Shah, Peshawar, Dr. Muhammad Sarwar, Faisalabad

**RECIPIENT OF
PROF. DR. A.R. SHAKOORI GOLD MEDAL 2018**



Dr. Iftikhar Ahmed

Principal Scientific Officer / Program Leader (NCCP), NARC, Islamabad.

Dr. Iftikhar Ahmed started his scientific career in 2001 as Scientific Officer in PARC, Islamabad. Later on, he went to University of Tokyo, Japan in 2003 and did his PhD degree in Microbial Biotechnology in 2007. He was promoted to Senior Scientific Officer and then Principal Scientific Officer in 2014. He has made tremendous scientific contribution in the subject field. The most important achievement to his credit is the identification of 25 novel species of bacteria (mentioned on next page), which have been validated by International Committee of Systematic in Prokaryotes (ICSP). Many of these novel species of bacteria has good potential of environment, bioremediation and plant growth promotion, etc. and published in international journals.

- ▶ Another important achievement on his credit is that he has the honor to publish the whole genome sequence of 11 boron-tolerant bacteria.
- ▶ In recognition to the discovery of the novel species of bacteria from Pakistan, a shield of honor was presented by Pakistan Society of Microbiology during the General Body Meeting in Lahore on 25th March, 2015.

*Other applicants of this award were Dr. Furhan Iqbal, Multan, Dr. Muhammad Akbar Khan, Lahore, Dr. Muhammad Irfan, Sargodha, Dr. Malik Hassan Mehmood, Karachi, Dr. Awais Ihsan, Sahiwal, Dr. Aamer Ali Shah, Islamabad, Dr. Muhammad Sajjad Ansari, Rawalpindi, Dr. Muhammad Naeem, Islamabad, Dr. Faiza Saleem, Lahore, Dr. Ghanzafar Ali, Muzaffarabad

- ▶ He has published more than 86 research papers in peer-reviewed journals of international repute with 1837 citations and more than 110 impact factor.
- ▶ He has also been awarded the Research Productivity Award by PCST since 2008 (Five Times).
- ▶ Based on his scientific achievements, he has also been awarded PARSA Science Award in 2014-15 in the open competition, Best Research Paper award.
- ▶ Presently, he is working as Program Leader of Microbial Culture Collection of Pakistan (NCCP), Islamabad.

**RECIPIENT OF
PROF. IMTIAZ AHMAD GOLD MEDAL 2018**



Dr. Riffat Sultana

Department of Zoology, University of Sindh, Jamshoro

Dr. Riffat Sultana earned her Ph.D in 2008 from University of Sindh, Jamshoro. She has described 14 new species and several new records of grasshoppers from sub-continent and to her credit there are 140 Published Research Papers in National and International journals of repute having 93.728 impact factor. She has been working on Biodiversity, Systematic, Biology, Ecology, Chemical & Biological Control and Food preference of Orthoptera including other orders of insects. She has produced 03 Ph.D and 09 M.Phil. She has been awarded by 03-Research Projects as Principal Investigator 02 by Higher Education Commission Islamabad and 01 by Pakistan Science Foundation Islamabad. She is Founder & Editor of University of Sindh Journal of Animal Sciences.

Dr. Sultana has also developed International Collaboration with University of Douala, Cameroon, University of Kelaniya Sri Lanka, Quanzhou Normal University, PR China and Gaziosmanpasa University Tokat, Turkey.

*Other applicant of this award was Dr. Zain ul Abdin, Faisalabad

**RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2018**



Dr. Shaukat Ali

Professor in the Department of Zoology, University of Azad Jammu and Kashmir,
Muza ffarabad

Dr. Shaukat Ali, Assistant Professor in the Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad obtained his M.Sc. (Hons.) degrees from the University of the Punjab, Lahore and Ph.D. degree from University of Leiden, The Netherlands. Due to his extraordinary Ph.D. thesis, he was awarded “Author of the best thesis of the year 2011” of Institute of Biology, Leiden University and he was also nominated author of the best thesis of the year 2011’ of Faculty of Sciences, and C.J. Kok prizes, Leiden University, The Netherlands.

Dr. Ali’s research is mainly in the field of Fish Medical Toxicology and specifically molecular mechanisms of Human diseases by using fish animal model. He has recently won a research project as Co-PI “Biodiversity, Antimicrobial Activity of Earthworm Species from Azad Jammu and Kashmir, Pakistan” From Higher Education of Pakistan. He has published over 45 original peer reviewed articles in journals.

*Other applicant of this award was Dr. Noor Khan, Pattoki

**RECIPIENTS OF
GOLD MEDALS AWARDED BY
THE ZOOLOGICAL SOCIETY OF PAKISTAN**

1. Muzaffer Ahmad Gold Medal 2018

The 25th Muzaffer Ahmad Gold Medal 2018 was received by Sania Nadeem a student of the University of the Punjab, Lahore for standing first in the recent M.Sc. Zoology examination.



Ms. Sania Nadeem

2. Afsar Mian Gold Medal 2018

Tenth Afsar Mian Gold Medal 2018 was given to Saba Mumtaz a student of the Arid Agriculture University, Rawalpindi for standing first in the recent M.Sc. Biology/Zoology examination.



Ms. Saba Mumtaz

3. Muhammad Afzal Hussain Qadri Memorial Gold Medal 2018

The 21st Muhammad Afzal Hussain Qadri Memorial Gold Medal 2018 was awarded to Fatima Hasan student of Karachi University for standing first in the recent M.Sc. Zoology examination.



Ms. Fatima Hasan

4. Prof. Dr. S.N.H. Naqvi Gold Medal 2018

The 14th Prof. Dr. S.N.H. Naqvi Gold Medal 2018 was given to Dr. Farkhunda Iftekhhar for obtaining Ph.D. degree in Zoology with specialization in the field of Toxicology from University of Karachi.



Dr. Farkhunda Iftekhhar

5. Mujib Memorial Gold Medal 2018

The 25th Mujib Memorial Gold Medal 2018 was given to Yasir Khan a student of Karachi University for standing first in the recent M.Sc. Zoology examination with specialization in Parasitology.



Mr. Yasir Khan

6. Prof. Dr. Muhammad Ali Gold Medal 2018

The 2nd Prof. Dr. Muhammad Ali Gold Medal 2018 was awarded to Beenish Javaid a student of Government College University, Faisalabad for standing first in the recent M.Sc. Zoology examination.



Ms. Beenish Javaid

7. Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2018

The 2nd Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2018 was awarded to Ahmed Yar a student of University of Sindh, Jamshoro for standing first in the recent final B.S. Examination of Freshwater Biology & Fisheries.



Mr. Ahmed Yar

8. Ahmed Mohiuddin Memorial Gold Medal 2018

The 13th Ahmed Mohiuddin Memorial Gold Medal 2018 was awarded to Ms. Tahira Noreen a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination.



Ms. Tahira Noreen

9. Prof. Dr. S.S. Akbar Memorial Gold Medal 2018

The 5th Prof. Dr. S.S. Akbar Memorial Gold Medal 2018 was awarded to Tahira Noreen a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination with specialization in Entomology.



Ms. Tahira Noreen

**AWARDED SHIELD AND CERTIFICATE
for his contributions as a Pioneer Geneticist in the field of Zoology**



Dr. Fazl-e-Majid Khan

Department of Zoology, University of the Punjab, Lahore

Dr. Fazle Majid Khan was born on April 28, 1931 in Bhera District Sargodha. Dr. Fazle Majid Khan then migrated with his parents to Multan where he completed his Primary education from Cantt Public School Multan Cantt in 1941. After getting Primary Education, he moved to Lahore and completed his elementary education from Watan Islamia High school Lahore in 1944 and Matriculation from S.D. Public School Empress Road Lahore in 1946. After completing his F.Sc. from Dyal Singh College Lahore in 1948, he got admission in Govt. College Lahore from where he obtained his B.Sc and M.Sc in Zoology in 1950 and 1953 respectively.

After getting Master degree in Zoology, he joined Govt. College Sahiwal as lecturer in October 1953 where he served till April 1956. From April 1956 – July 1956 he served in Department of Biology, Govt. Postgraduate College Chakwal as Lecturer and then transferred to Govt. College Faisalabad. After serving for about 3 years (July 1956-Oct 1959) in Govt. College Faisalabad, Dr. Fazle Majid Khan joined Govt. College Lahore where he served as Lecturer Biology from 1959 to February 1962.

In Feb 1962, Dr. Fazle Majid Khan moved to Australia and joined University of Queensland Brisbane Australia as Research Scholar and obtained his Doctoral Degree in Genetics in 1966. Dr. Fazle Majid Khan has honor to work as Secretary/Treasurer of Genetics Society of Queensland Brisbane from 1963-1966.

After obtaining his Doctoral Degree, he came back to Pakistan and Joined Department of Zoology, University of the Punjab, Lahore in August 1966 as Faculty member. In October 1981, Dr. Fazle Majid Khan joined faculty at University of Maiduguri Nigeria. In September 1984, Dr. Fazle Majid Khan left University of Maiduguri and joined National Academy for Higher Education UGC Islamabad and then worked as Regional Director UGC, Lahore. Later on, Dr. Fazle Majid Khan worked as R.O.1 in Punjab University Lahore from September 1985 to April 1991. Dr. Fazle Majid Khan retired from Department of Zoology University of the Punjab, Lahore as Associate Professor on April 27, 1991.

Owing to his exceptional expertise in classical as well as in population genetics, he has been requested several times for teaching genetics at BZU, Multan, A.J.K. University, Muzzafarabad and Punjab University, Lahore. Dr. Fazle Majid Khan has honor to work as Secretary/Treasurer of Genetics Society of Queensland Brisbane from 1963-1966.

**AWARDED SHIELD AND CERTIFICATE
for his contributions towards promotion of Zoological Society in his
capacity as Founder Member of ZSP**



Prof. Dr. Akbar Ali Khan

Ex-Chairman, Department of Zoology & Fisheries,
University of Agriculture, Faisalabad

Dr. Akbar Ali Khan did his M.Sc. in 1965 from Department of Zoology, University of the Punjab, Lahore. He rendered valuable services to the zoological society of this Department as president during the year 1964-65. He joined the Department of zoology, University of Agriculture, Faisalabad in Nov., 1966 and retired as Professor and Chairman in February 2002. He obtained Ph.D. in zoology in 1982 from University of Agriculture, Faisalabad.

He is the Founder fellow. (1968) and Life Fellow of the Zoological society of Pakistan (International). He has been elected as unopposed member of Executive council of the society twice.

Congress of Zoology (International) was held three time sat University of Agriculture, Faisalabad, once in 1982, then in 2001 and the latest in 2010.

He was the chairman of the local organizing committee of the congress held in 2001.

He actively participated in all the above-mentioned congress. He perused hundreds of his students to enroll as fellow/members of the society and one of his students conducted the 30th congress of zoology (International) held in 2010 at University of Agriculture, Faisalabad as chairperson of the local organizing committee. He had been member of the Editorial Board of Pakistan Journal of zoology for many years.

Some Glimpses of Academic Sessions and the Congress Participants























FUTURE TRENDS FOR SUSTAINABLE AQUACULTURE IN TURKEY

TELAT YANIK

*Ataturk University, Faculty of Aquaculture, Department of Fishery,
25240-Erzurum, Turkey*

Abstract.- It is aimed to overview status, sustainable studies and future trends of aquaculture sector by prediction using official data in this paper. Aquaculture is considered as a global industry to provide food and an independent sector and represented by General Directorate of Fisheries and Aquaculture under Ministry of Agriculture and Forestry (MAF) in Turkey. Following plant production, animal husbandry and forestry, fisheries are considered one of four sub sectors (2.7%) of agricultural production with 0.3% in GNP (Gross National Product). Aquaculture activities have been applied in earthen and concrete ponds, fiberglass tanks and in cages in commercial and family production type farms since 1970. Nowadays, additional to land based farms, trout production can be seen in cages both in inland waters and sea waters. Many hatcheries and feed production companies as well as technological supply companies have been established with the improvement of aquaculture sector and aquaculture education at universities in Turkey. Aquaculture production was 0,23% of the total production of the world in 2016. It is stated that aquaculture production will increase 47.02 % in 2023 and became 500,000 tons with a 1,125,000,000 US dollar commercial value by the State Planning Organization. Along with the development of aquaculture, some environmental problems were reported from water sources. According to the equation, the total production of Turkey will be 500.000,00 tons nearly by 2035. It is concluded that future studies are required by using new technologies after evaluating of past studies conducted on many fish species.

Keywords: Turkey, aquaculture production, fish farming, sustainability and environmental impacts

INTRODUCTION

Farmed fish industry is growing rapidly and provides approximately 50% of the global food supply (FAO, 2016). It is predicted that the population of the world will 9 billion and thus aquaculture outcome should be increased 100% until 2050 in order to provide adequate food to people. It is expected that people will consume a lot of cultured fish in the future. Compared to large animal production (63 million tonnes), aquaculture production (66 million tonnes) is higher, but it has negative effects on the environment due to reared fish species in 2012. Culture of herbivore and detritivore fish species are considered environmentally friendly and took the biggest part of the production, for example, China has 62% of the world production and deals with carps.

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Turkey has a great deal of aquaculture potential with 8333 km coastline, 24.607.200 ha surface area of seas and 200 natural lakes with 906.118 ha, 159 dam lakes with 342.377 ha, 750 ponds with 15.500 ha and 33 rivers with 177.714 km lengths (Kayhan and Olmez, 2014). Aquaculture is based on the Regulations of Fisheries published on 29 June 20014 with 25507 number in Turkish Official Gazette (T.C. Resmi Gazete) and governed independently by General Directorate of Fisheries and Aquaculture (BSGM) under the Ministry of Food, Agriculture and Livestock (MoFAL) (Balci Akova, 2015; MoFAL, 2018).

With the improvement of aquaculture sector, aquaculture education in universities, many hatcheries and feed production companies as well as technological supplies such as nets and fiberglass tanks have been established in Turkey. There are 11 faculties and 25 high school for aquaculture in Turkey. Baki and Yucel (2016) analyzed economically amount of rainbow trout, sea bass and sea bream between 2005 and 2014. They have concluded that kg prices of fish species reduced gradually, %43 in rainbow trout, 25% in sea bass and 41 % in sea bream from 2005 to 2014. Total price of sea bass was leading with 402.22 million USD in 2012. It shows that the more production increases the price gets lower. However, increasing production volume causes also serious environmental problems in water sources, as an example, Sasi *et al.* (2017) reported high pollution rate due to sea bream and sea bass farming in Bafa lake. Along with the development of aquaculture, some environmental problems were reported from water sources. Only one sustainable fish farm, Pınar was reported so far. There have been some studies dealing with the SWOT analysis of aquaculture of Turkey or summarizing situation with less scientific data due to its progress of improvement rate and so with less assumption in the past. Most of them provides many useful old historical data. Therefore, it is aimed to overview about the latest status and future trends for sustainable and responsible aquaculture with constraints and promises in Turkey. The present paper can also be considered as sample for the other countries to test their current performance and make detailed plans and programs for future.

MATERIAL DATA

Aquaculture data from Turkish Statistics Institute, FAO, Turkish Ministry of Agriculture and Forestry formerly known as Turkish Ministry of Food Agriculture and Livestock, World Fish Center have been collected. A growth graph was drawn by using old data starting from 2000 in order to determine the relationship between years and growth rate of Turkey. Equations were tested for

the best fitting of the graph line. After determination of the best equation, future aquaculture productions were predicted by using statistical software. Many other articles published internationally have been also gathered for the evaluation.

RESULTS AND DISCUSSION

It is reported that there are 26 fish families with 236 fish species and sub species in inland waters of Turkey (FAO, 2018). Most of them are from the same family, i.e. 116 species from cyprinid family and their economic value are low, some of them, 102 species, are listed in red lists of The International Union for Conservation of Nature (IUCN), 29 migratory species are listed in Bern Convention List created in European Wildlife and Natural Habitats Conservation Convention by the European Council (1979). Finally, 6 endangered species have been listed both in the Bern and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Considering these facts, any aquaculture production activity should consider these facts or give priorities on these species to save them or restore again natural water resources in addition to economical concern. Introducing new species into aquaculture for such purposes in the world as well as in Turkey have been tried to do (Yanik *et al.*, 2009).

On the other hand, there has been various studies for maximum production by using new feed stuffs or replacing alternative feed stuffs into fish diets experimentally. Such studies focused mainly on replacement of fish meal due to limited availability and price (Yanik and Aras, 1999).

Status of aquaculture in Turkey

Interest in fishing might be a very old practice backs to ancient mythological Romanian times in Anatolia. Since some mosaic frescos which included many sea fish species have been discovered in excavations and exhibited in Zeugma Museum in Gaziantep city *e.g.* in Figure 1 in Turkey (Anonymous, 2018).

Harlioglu (2011) reported that Anchovy (*Engraulis encrasicolus*) from black sea and an indigenous carp (*Chalcalburnus tarichii*) from brackish water of Van Lake and common carp from inland waters are the main catchment species of Turkey. Total quota for tuna from the Mediterranean Sea was 22.695 tons in 2017 and for the years 2018, 2019 and year 2020 is decided to increase to 28.000, 32.000 and 36.000 tons respectively. Turkey's quota is increased by 40% that means its catchment share will increase from 4.4% to 6.22% in 2020, while the

other countries will not change based on the decision made by the International Commission for the Conservation of Atlantic Tunas (ICCAT) in the last meeting in Marrakech in Morocco (ICCAT, 2017).



Fig. 1. Poseidon, Oceanos and Tethys Mosaic (Anonymous, 2018)

Turkey took second place after Norway in EU with respect to aquaculture production of seabass and seabream and of rainbow trout. Aquaculture production was 79,031 tons in 2000 and it became 235,133 tons (48.31% rainbow trout, 31.75% sea bass and 17.81% sea bream) in 2014 (Baki and Yucel, 2016). It reached 253,395 tons which was 43,04% of total fishery production of Turkey and 0,23% of the total aquaculture production of the world in 2016. It is reported that 18% of 2326 number of total fish farms are sea based with 60 % of total aquaculture production (MoFAL, 2018). Total aquaculture production of Turkey is presented in Figure 2. It is stated that aquaculture production will increase 47.02 % in 2023 and became 500,000 tons with a 1,125,000,000 US dollar commercial value by the State Planning Organization of Turkey (MoFAL, 2018).

In Europe, Turkey ranks 6th in fish farming production and 3rd among Caucasian countries. Following plant production, animal husbandry and forestry, fisheries are considered one of four sub sectors (2.7%) of agricultural production with 0.3% in GNP (Gross National Product) (OECD, 2008).

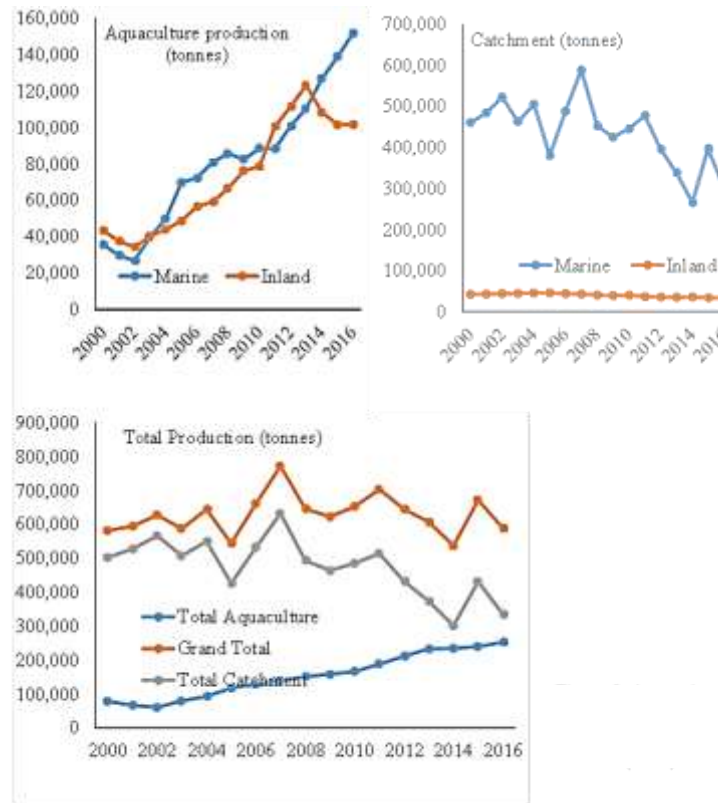


Fig. 2. Aquaculture production of Turkey (data provided by mOFAL (2018))

Species for aquaculture

Fish farming was started at 1970s. It was a rainbow trout farm (*Oncorhynchus mykiss*) at first then followed by gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) after mid of 1980s and tuna took place in 2000s (Memis *et al.*, 2002). Carp (*Cyprinus carpio*) and Tilapia (*Oreochromis niloticus*) are also farmed but not at big quantities. Nowadays, additional to land based farms trout production can be seen in cages both in inland waters and sea waters. Marine and fresh water fish species have been produced in off shore cages and earthen ponds as well as in fiberglass tanks in modern facilities such as sturgeons (*Acipenser* spp.), black sea trout (*Salmo trutta*) and turbot (*Psetta maxima*) in Black sea region. Bluefin tuna (*Thunnus thynnus*) and Mediterranean mussel (*Mytilus galloprovincialis*) has been cultured in other regions of Turkey.

Researchers have been focused on either introducing of some new fish species with economic value or using alternative species into aquaculture sector in inland and sea water sources. In the last decade, new studies have been conducted on commercial aquaculture production dealing with alternative fish species such as common seabream (*Pagrus pagrus*), sharpsnout seabream (*Puntazzo puntazzo*), white grouper (*Epinephelus aeneus*), corb (*Umbrina cirrosa*), meagre (*Argyrosomus regius*), brown meagre (*Sciaenops ocellatus*), white seabream (*Diplodus sargus*), two-banded seabream (*Diplodus vulgaris*), common dentex (*Dentex dentex*), common pandora (*Pagellus erythrinus*), striped seabream (*Lithognathus mormyrus*) and greater amberjack (*Seriola dumerili*) (FAO, 2018).

Koksal *et al.* (2000) reared siberian sturgeon (*Acipenser baeri*) in concrete ponds successfully and suggested that this can be reared rainbow trout fish farms and easy to farm such as sea bass and sea bream. Common dentex (*Dentex dentex* L.) was reared alternatively to sea bass and sea bream for aquaculture (Cakli *et al.*, 2005). Turkmen (2007) studied culture of shrimp (*Penaeus semisulcatus*) in Aegean Sea. Yanik *et al.* (2009) tried to take indigenous barbel (*Barbus capito capito*) under culture with no success. Coban *et al.* (2009) investigated growth stages of red porgy (*Pagrus pagrus*) in a controlled hatchery for aquaculture. Sharp-snout sea bream (*Diplodus puntazzo*) and common dentex (*Dentex dentex* L.) aquaculture, however, caused some parasitic problems in fish produced intensively in cages (Toksen, 2006; Toksen and Cilli, 2010). Bulut *et al.* (2014) investigated introduction of two-banded seabream (*Diplodus vulgaris*) into aquaculture with a feeding study with positive results.

Sustainability studies

It is suggested that technological improvements are needed for the sustainability and studies must continue on breeding and genetics methods, control of disease with combination of new technologies for example a system with the diagnosis and vaccine ability at the same time, feeding techniques, quality of feed and environment friendly products from healthy systems (Waite *et al.*, 2014).

Yanik and Aras (1999) found that 50 % slaughterhouse by products can be used in rainbow trout, *Oncorhynchus mykiss*, diets. Aydin and Gumus (2013) reported same percentage after replacement of poultry by-product meal in Nile

tilapia fry, *Oreochromis niloticus*. Gumus *et al.* (2009) reported a 20% replacement with tuna liver meal in carp fry diets.

Eroldogan *et al.* (2006). evaluated some feeding protocols in sea bream, *Sparus aurata* for the purpose of better feed conversion ratio. Merrifield *et al.* (2010) investigated the usage possibility of *Chlorogloeopsis* in the diets to increase production of Nile tilapia. Gultepe *et al.* (2011) used Bio-Mos in diets to increase growth of gilthead sea bream. Korkmaz *et al.* (2011) used dried baker's yeast for the same purpose and found 30% replacement rate in koi fish, *Cyprinus carpio*. In mirror carp, *Cyprinus carpio*, there has been also studies with positive results to increase productivity (Yigit *et al.*, 2013). Koprucu (2012) conducted a research on protein and energy digestibility by using local feed stuffs in grass carp, *Cyprinus carpio*. Cogun and Sahin (2012) studied effects of zeolite to prevent the negative effects of environmental conditions in Nile tilapia, *Oreochromis niloticus* and in common carp, *Cyprinus carpio* by Mutlu *et al.* (2016). Karahan *et al.* (2013) tried to suggest a way to increase production of European sea bass by using genetic detection of deformities at early stages. Ercan *et al.* (2015) studied the effects of salinity levels of effluent waters on growth European sea bass.

Some feed additives have also been used for technology development for example 100g /kg kefir addition advised to increase meat quality in rainbow trout (Gumus *et al.*, 2017). Turkmen *et al.* (2017) used micro diets to increase larval growth and survival rates of larvae of gilthead seabream. Mannan-oligosaccharide (MOS) has been tested in diets to increase growth of gilthead sea bream (Gultepe *et al.*, 2011, 2015; Gelibolu *et al.*, 2018).

Aquaculture products have been processed and some technologic studies were conducted to increase total income. Erkan (2007) worked on the meat quality properties of sea bass and sea bream. Basaran *et al.* (2007) suggested using offshore cages to have less impact on the environment after monitoring production areas of sea bream and sea bass in cages in Ildir Bay. Since the main concern is mostly income other than environmental issues, pollution has been becoming a serious issue in waters of Turkey. Yucel-Gier *et al.* (2009) mentioned that improvement of aquaculture caused environmental problems to be solved either by precautions or legislations. Deniz and Benli (2009) reported that many marine facilities were closed due to wrong site selection where archeological sites exist.

Future trends of aquaculture in Turkey

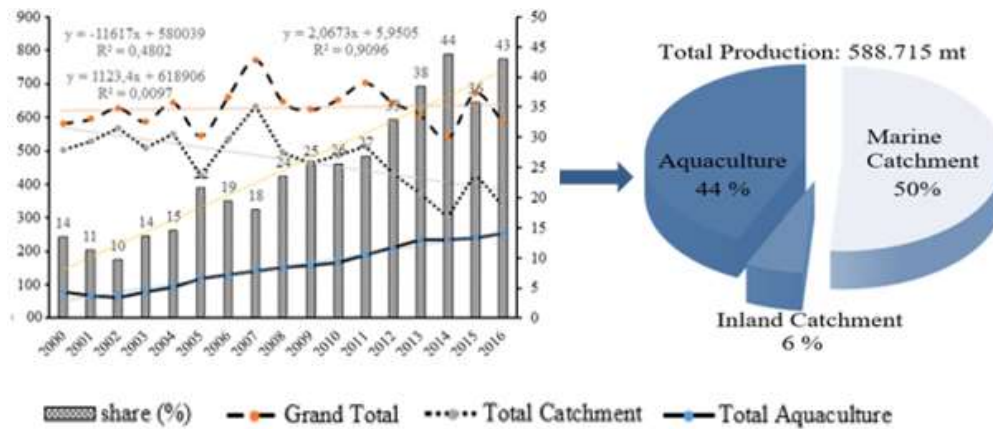
Turkey is a self-sufficient country from many aspects especially food supply. Using effectively all natural resources will increase its strength economically in the world. For example, total tuna catchment quota of Turkey will increase from 1010.43 tons in 2017 to 1414 in 2018, 1824 in 2019 and 2240 tons in year of 2020. With this latest decision, the export amount of Turkey is projected from 100.000.000 to 130.000.000 US dollars for year of 2020 (ICCAT, 2017).

Aquaculture is the fourth sector, which includes cage culture and land based production in Turkey. There are 3 sub sectors of fisheries: Inland, recreational and aquaculture. Aquaculture production was 79,031 tons in 2000 and it became 235,133 tons in 2014 and finally it reached 253,395 tonnes in 2016. Growth rate of the aquaculture is therefore only 4,2 % which is lower than that of the world's average. It is reported that a 24% of increase in total fish production was recorded from 2006 to 2015 with a 50% consumption rate of aquaculture production. It is expected that consumption rate will be increased to 57% but growth rate of aquaculture will drop from 5,4 to 3% annually in 2025 (OECD/FAO., 2017a; OECD/FAO, 2017b).

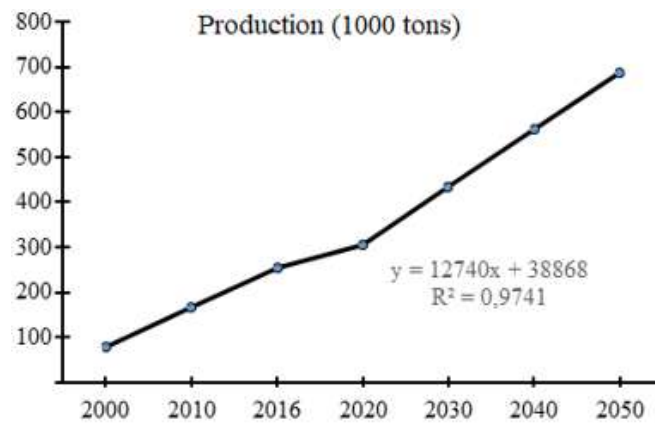
It can be seen from Fig 3a, the share of aquaculture and catchment rate in total fisheries production of Turkey in 2016. Total aquaculture took place 44% and catchment is 56% in the year of 2016, but MoFAL (2018) declared that there will be 500000 tonnes of aquaculture production by the years of 2023. However, statistical predictions show that it is far too catch this volume with the same increasing rate so far achieved (Fig. 3b).

Considering the water potential and suitable places for aquaculture of Turkey, it can be said that the aquaculture production from inland and seawater is still low and should be increased. Sustainability and using technology is the best policies for maximum fish production. As it is projected almost double or triple for Turkey, the production should be sustainable and use the sources effectively with environment friendly policies. Product variety should be increased and consumption should be increased by commercials. Since, fish consumption is depending on the economical income and traditional eating habits. For example, in East or southeastern regions, consumption rate is very low, about 800 g⁻¹ kg per capita/year. Food should be marketed safely and freshly. The main disadvantages are lack of processing facilities, almost 75 % of marine and nearly

100 % fresh water products has been consumed as fresh. Therefore, marketing is not enough, 65 % of the total products is wholesaled and 1% is for the processing. Most of the fishing vessels (more than 80 %) has no cooling system.



(A)



(B)

Fig. 3. Total fisheries production and related share (A) and shares and expectation of aquaculture production (B) in Turkey by 2050 ($y = 12740x + 38868$, $R^2 = 0.9741$) (data used from MoFAL, 2018).

Potentially, based on the current data, it seems Turkey use less than half of its resources suitable to aquaculture. Therefore, aquaculture production can be easily increased by either using more sources or increasing technology to produce feed with low pollutant rate as well as using disease free certified brood stocks. It is reported that there have been some pollution problems in Turkey due to aquaculture (Deniz, 2001) as the stock rate increase the pollution increase. When stock amount increase in unit area it might be good for high production, but requires much more water volume which means extra energy and high quality feeds with higher protein and energy values which may cause water pollution due to high waste rate. Intensive aquaculture with excess stock rate is always risky for disease outbreaks.

Considering sustainability, there should be careful monitoring and precautions (Deniz and Benli, 2009). Technological improvements may help for this purpose as they provide information to produce disease resistant and high growth rate strains with genetic methods (Karahana *et al.*, 2013) They also give opportunity for producers or researchers to produce high quality feeds with alternative feed stuffs (Koprucu, 2012; Gumus *et al.*, 2017). Another advantage of using technology may allow the producers to joint productions to increase production by recirculating systems, aquaponics (Addy *et al.*, 2017; Nuwansi *et al.*, 2017; Pinho *et al.*, 2018) and bio floc systems in various species (for African catfish by Dauda *et al.*, 2017; for Nile tilapia by de Alvarenga *et al.*, 2017; Jung *et al.*, 2017; in common carp by Bakhshi *et al.*, 2018; for *Tinca tinca* and grey *Mugil cephalus* by Vinatea *et al.*, 2018) and hydroponic systems which have been considered as high productivity with low pollution systems. Considering above activities in the world, it crucial to start integrated fish farming with poultry or beekeeping in Turkey also. It will contribute to creating job opportunities in rural areas and preventing migration. It is also required to develop and introduce new aquaculture systems with new species and new technological methods in freshwater and salt water systems.

Although there has not been any activity related technological production systems other than recirculating systems in aquaculture of Turkey, it is projected that targeted aquaculture production can be realized either by contribution of using new technologies (Deb *et al.*, 2017) or by introducing new species such as shrimp (Maciel *et al.*, 2018; Pujiastuti and Suwartha 2018), and piracanjuba, *Brycon orbignyanus* (Sgnaulin *et al.*, 2018) into production programs similarly to the other developed countries did in the world. No crustaceans or mollusk commercial farms yet established in Turkey. There is one tilapia farm with

technological equipment. Therefore, it is projected that there is a big potential for Turkey to increase its sustainable production.

CONCLUSIONS

It is the fact that Turkey has strengths, weaknesses, opportunities, and threats for the sustainable and responsible of aquaculture. The future projections show that the aquaculture production will be increased and it will keep its place (27th in rank) in the world. Since, Turkey has advantages with its 5 km² still waters and rivers 175.714 km rivers with rich aquatic fauna. Wild fish species with economic value need to be taken under culture. Red paper work and tedious procedures for importing and exporting and licensing for aquaculture can be considered weakness and should be reduced. Feed quality and availability would be considered as another restriction and should be improved. Some of the main threads to be healed may be listed as pollution, competition with tourism sector, competition with hydro-energy sector and competition power with big companies in EU.

Turkey is still improving technology to use its sources at maximum rate without disturbing natural fish populations. It is predicted that the resources of the world allocated to fisheries and aquaculture will be constant with its maximum usage rate in near future. Turkey is promising for investors with its cheap labor force and abundant decent places for aquaculture. However, labor might be a problem in the aquaculture sector, since it has been observed a serious migration problem so far from rural areas to city centers; for example, there are many villages almost no inhabitants during winter time in Northern Anatolia Region.

New technological methods should be used for increasing feed evaluation (lower FCR). Number of intensive fish farms with maximum stock rates should be increased for sustainable production. It is concluded that recirculating systems and joint or integrated production farms must be constructed to meet the future demands of food to prevent shortage in future.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Prof. Dr. Abdul Rauf Shakoory, Distinguished National Professor and Director, School of Biological Sciences,

Emeritus Professor from University of the Punjab for giving the idea and making very valuable comments on this paper.

REFERENCES

- ADDY, M. M., KABIR, F., ZHANG, R. C., LU, Q., DENG, X. Y., CURRENT, D., GRIFFITH, R., MA, Y. W., ZHOU, W.G., CHEN, P. AND RUAN, R., 2017. Co-cultivation of microalgae in aquaponic systems. *Bioresour Tech.*, **245**: 27-34 DOI: 10.1016/j.biortech. Gultepe, N., Karga, requirements of two-banded seabream (*Diplodus vulgaris*) cultured in a recirculating aquaculture system. *Aquacul. Int.* **22**: 965-973. DOI: 10.1007/s10499-013-9720-z.2017.08.151 Part: A.
- ANONYMOUS, 2018. *Zeugma museum*. <http://www.zeugma.org.tr/hikayeler.aspx#features/6> (Entered: January 6, 2018).
- AYDIN, B. AND GUMUS, E., 2013. Replacement of Fishmeal by Poultry By-Product Meal, Supplemented with Lysine, Methionine, and Threonine, in Diets for Fry of Nile Tilapia (*Oreochromis niloticus*). *Israeli J. Aquacult. -Bamidgeh*, IIC:65.2013.885, 7p.
- BAKHSHI, F., NAJDEGERAMI, E.H., MANAFFAR, R., TUKMECHI, A. AND FARAH, K.R., 2018. Use of different carbon sources for the biofloc system during the grow-out culture of common carp (*Cyprinus carpio* L.) fingerlings. *Aquaculture*, **484**: 259-267. DOI: 10.1016/j.aquaculture.2017.11.036.
- BAKI, B. AND YUCEL, S., 2016. Production volume, price and value analyses of Turkish aquaculture products. *Int J. Ecosyst. Ecol. Sci.*, **6**: 431-436.
- BALCI AKOVA, S., 2015. Aquaculture and its distribution in Turkey. *J. Aquacul. Engin. Fish. Res*, **1**:160-190. DOI: 10.3153/JAEFR15018.
- BASARAN, A.K., AKSU, M. AND EGEMEN, O., 2007. Monitoring the impacts of the offshore cage fish farm on water quality located in Ildir Bay (Izmir-Aegean Sea). *J. agric. Sci*, **13**: 22-28.
- BULUT, M., YIGIT, M., ERGUN, S., KESBIC, O.S., ACAR, U., CAKLI, S., DINCER, T., CADUN, A., FIRAT, K. AND SAKA, S., 2005. Quality characteristics of wild and cultured common dentex (*Dentex dentex*, Linnaeus, 1758). *Arch. Lebensmittelhyg.*, **56**: 104-108.
- COBAN, D., SUZER, C., KAMACI, H.O., SAKA, S., AND FIRAT, K., 2009. Early osteological development of the fins in the hatchery-reared red porgy, *Pagrus pagrus* (L. 1758). *J. appl. Ichthyol*, **25**: 26-32. DOI: 10.1111/j.1439-0426.2008.01165.x.
- COGUN, H.Y. AND SAHIN, M., 2012. The Effect of Zeolite on Reduction of Lead Toxicity in Nil Tilapia (*Oreochromis niloticus* Linnaeus, 1758). *Kafkas Univ. Vet. Fakul. Derg.*, **18**: 135-140.
- DAUDA, A.B., ROMANO, N., EBRAHIMI, M., KARIM, M., NATRAH, I., KAMARUDIN, M.S. AND EKASARI, J., 2017. Different carbon sources affect biofloc volume, water quality and the survival and physiology of African catfish, *Clarias gariepinus*, fingerlings reared in an intensive biofloc technology system. *Fish. Sci*, **83**: 1037-1048. DOI: 10.1007/s12562-017-1144-7.
- De ALVARENGA, E.R., DE SALES, S.C.M., DE BRITO, T.S., SANTOS, C.R., CORREA, R.D.S., ALVES, G.F.D., MANDUCA, L.G. AND TURRA, E.M., 2017. Effects of biofloc technology on reproduction and ovarian recrudescence in Nile tilapia. *Aquacul. Res*, **48**: 5965-5972. DOI: 10.1111/are.13420.

- DEB, S., NOORI, M.T. AND RAO, P.S., 2017. Experimental study to evaluate the efficacy of locally available waste carbon sources on aquaculture water quality management using biofloc technology. *Aquacul. Int.*, **25**: 2149-2159. DOI: 10.1007/s10499-017-0180-8.
- DENİZ, H., 2001. Environmental impact of aquaculture in Turkey and its relationship to tourism, recreation and sites of special protection. In: (eds. C. A. Zaragoza and U.B. Basurco) *Environmental impact assessment of Mediterranean aquaculture farm. Cahiers Options Méditerran.*, **55**: 159-171.
- DENİZ, H. AND BENLİ, A.C.K., 2009. Environmental policies in force and its effects on aquaculture in Turkey (ed. Ozhan E.) Second International Conference/Workshop on the State-of-the-Art of ICM in The Mediterranean and Black Sea: Immediate Needs for Research, Education / Training and Implementation. 14-18 October 2008, Akyaka, Turkey Pages: 193-+
- ERCAN, E., AGRALI, N. AND TARKAN, A.S., 2015. The effects of salinity, temperature and feed ratio on growth performance of European sea bass (*Dicentrarchus labrax* L., 1758) in the water obtained through reverse osmosis system and a natural river. *Pakistan J. Zool.*, **47**: 625-633.
- ERKAN, N., 2007. Freshness and quality of aquacultured sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) stored in ice. *Archi. Lebensmittelhyg.*, **58**: 98-106. DOI: 10.2377/0003-925X-58-98.
- EROLDOĞAN, O.T., KUMLU, M., KIRIS, G.A. AND SEZER, B., 2006. Compensatory growth response of *Sparus aurata* following different starvation and refeeding protocols. *Aquacul. Nutri.*, **12**: 203-210 DOI: 10.1111/j.1365-2095.2006.00402.x.
- FAO, 2018. *Fishery country profile, fishery sector structure of Turkey* (March, 2008). http://www.fao.org/fishery/docs/DOCUMENT/fcp/en/FI_CP_TR.pdf, Last entered 11/02/2018.
- GELİBOLU., YASEMEN, Y., AYCE, G. AND ERCUMENT, G., 2018. Effect of mannan-oligosaccharide supplementation on body growth, fatty acid profile and organ morphology of gilthead seabream, *Sparus aurata*. *Pakistan J. Zool.*, **50**: 229-240, DOI: <http://dx.doi.org/10.17582/journal.pjz/2018.50.1.229.240>.
- GULTEPE, N., SABRI KESBİÇ, O., ACAR, Ü., GÖKKUŞ, K., GÜLTEPE, M.İ., SÖNMEZ, A.Y., BİLEN, S. AND AYDIN, S., 2015. Effects of prebiotic mannanoligosaccharides (MOS) on histology and biochemical blood parameters of gilthead seabream, *Sparus aurata*. *Israeli J. Aquacul - Bamidgeh, IJA*, **67**.2015.1072, pp. 6.
- GULTEPE, N., SALNUR, S., HOSSU, B. AND HISAR, O., 2011. Dietary supplementation with Mannanoligosaccharides (MOS) from BioM., Yilmaz, S., and Guroy, D., 2014. Evaluation of dietary protein and lipid-Mos enhances growth parameters and digestive capacity of gilthead sea bream (*Sparus aurata*). *Aquacul. Nutri.*, **17**: 482-487 DOI: 10.1111/j.1365-2095.2010.00824.x.
- GUMUS, E., KAYA, Y., BALCI, B.A. AND ACAR, B.B., 2009. Partial replacement of fishmeal with tuna liver meal in diets for common carp fry, *Cyprinus carpio* L. 1758. *Pakistan Vet. J.*, **29**: 154-160.
- GUMUS, E., KUBILAY, A., GUNEY, S., GUZEL-SEYDIM, Z., KOK-TAS, T., METIN, S. AND ULUKOY, G., 2017. Effect of dietary kefir on the growth performance, feed utilization and fatty acid profile of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquacul. Nutri.*, **23**: 964-972. DOI: 10.1111/anu.12464.
- HARLIOĞLU, AG., 2011. Present status of fisheries in Turkey. *Rev. Fish Biol. Fish.*, **21**: 667-680. DOI: 10.1007/s11160-011-9204-z.

- ICCAT, 2017. *25th Regular Meeting of the International Commission for the Conservation of Atlantic Tunas* (ICCAT). Marrakech, Morocco, 14 to 22 November 2017.
- JUNG, J.Y., DAMUSARU, J.H., PARK, Y., KIM, K., SEONG, M., JE, H.W., KIM, S. AND BAI, S.C., 2017. Autotrophic biofloc technology system (ABFT) using *Chlorella vulgaris* and *Scenedesmus obliquus* positively affects performance of Nile tilapia (*Oreochromis niloticus*) *Algal Res. Biomass Biofuels Bioprod.*, **27**: 259-264. DOI: 10.1016/j.algal.2017.09.021.
- KARAHAN, B., CHATAIN, B., CHAVANNE, H., VERGNET, A., BARDON, A., HAFFRAY, P., DUPONT-NIVET, M., AND VANDEPUTTE, M., 2013. Heritabilities and correlations of deformities and growth-related traits in the European sea bass (*Dicentrarchus labrax*, L) in four different sites. *Aquacul. Res.*, **44**: 289-299. DOI: 10.1111/j.1365-2109.2011.03082.x.
- KAYHAN, M.H., AND OLMEZ, M., 2014. Aquaculture and organic aquaculture in Turkey. *J. Aquacul. Res. Develop.*, **5**: 259. doi:10.4172/2155-9546.1000259.
- KOKSAL, G., RAD, F. AND KINDIR, M., 2000. Growth performance and feed conversion efficiency of Siberian sturgeon juveniles (*Acipenser baeri*) reared in concrete raceways. *Turkish J. Vet. Anim. Sci.*, **24**: 435-442.
- KOPRUCU, K., 2012. Variability in the chemical composition of different cottonseed and sunflower meals influences their digestibility when fed to grass carp (*Ctenopharyngodon idella*). *Aquacul. Nutri.*, **18**: 662-672 DOI: 10.1111/j.1365-2095.2011.00929.x.
- KORKMAZ, A.S., KORKMAZ, A.S. AND CAKIROGULLARI, G.C., 2011. Effects of partial replacement of fish meal by dried baker's yeast (*Saccharomyces cerevisiae*) on growth performance, feed utilization and digestibility in koi carp (*Cyprinus carpio* L., 1758) fingerlings. *J. Anim. Vet. Adv.* 10. 346-351. 10.3923/javaa.2011.346.351.
- MACIEL, J.C., FRANCISCO, C.J. AND MIRANDA, K.C., 2018. Compensatory growth and feed restriction in marine shrimp production, with emphasis on biofloc technology. *Aquacul. Int.*, **26**: 203-212 DOI: 10.1007/s10499-017-0209-z.
- MEMIS, D., DEMIR, N., EROLDogan, O.T. AND KUCUK, S., 2002. Aquaculture in Turkey. *Israeli J. Aquacul-Bamidgeh*, **54**: 34-40.
- MERRIFIELD, D.L., GUROY, D., GUROY, B., EMERY, M.J., LLEWELLYN, C.A., SKILL, S. AND DAVIES, S.J., 2010. Assessment of *Chlorogloeopsis* as a novel microbial dietary supplement for red tilapia (*Oreochromis niloticus*). *Aquaculture*, **299**: 128-133. DOI: 10.1016/j.aquaculture.2009.12.004.
- MoFAL, 2018. Ministry of Food Agriculture and Livestock - General Directorate of Fisheries and Aquaculture. *Official Fisheries Statistics for aquaculture production and freshwater products*. (in Turkish: Gıda Tarım ve Hayvancılık Bakanlığı, Balıkçılık ve Su Ürünleri Genel Müdürlüğü (BSGM). *Su Ürünleri İstatistikleri*. Available at: <https://www.tarim.gov.tr/sgb/Belgeler/SagMenuVeriler/BSGM.pdf>.
- MUTLU, E., AYDIN, S., DEMIR, T., AND YANIK, T., 2016. Effect of zeolite and copper sulfate, administered alone and in combination on the biochemical components of blood serum of common carp, *Cyprinus carpio*. *Pakistan J. Zool.*, **48**: 1857-1863.
- NUWANSI, KKT., VERMA, AK., TIWARI, VK., PRAKASH, C. AND CHANDRAKANT, MH., 2017. Standardization of the stocking density ratios of koi carp (*Cyprinus carpio* var. koi): goldfish (*Carassius auratus*) in polyculture aquaponic recirculating system. *Turkish J. Fish. aquat. Sci.*, **17**: 1271-1278 DOI: 10.4194/1303-2712-v17_6_20.

- O.E.C.D, 2008. *Country note on national fisheries management systems*, Turkey. Accessible at: <https://www.oecd.org/turkey/34431494.pdf>.
- OECD/FAO. 2017a. *Agricultural Outlook 2017–2026, Fish and Seefood*. OECD-FAO Food and Agriculture Organization of the United Nations, Paris, France.
- OECD/FAO. 2017b. *Agricultural Outlook*, OECD Agriculture Statistics (Database). Food and Agriculture Organization of the United Nations, Rome, Italy.
- PINHO, S.M., de MELLO, G.L., FITZSIMMONS, K.M. AND EMERENCIANO, M.G.C., 2018. Integrated production of fish pacu, *Piaractus mesopotamicus*, and red tilapia *Oreochromis sp.* with two varieties of garnish (*scallion* and *parsley*) in aquaponics system, *Aquacul. Int*, **26**: 99-112 DOI: 10.1007/s10499-017-0198-y.
- PUJASTUTI, DR. AND SUWARTHA, N., 2018. Enhancing removal efficiency of ammonia and nitrate in shrimp farm wastewater using biofloc technology and effective microorganism S4 (EM4). *Int. J. Technol.*, **8**: 1021-1030. DOI: 10.14716/ijtech. v8i6.685.
- SASI, H., DEMIR, H., AKZIYPAK, R. AND SAIDU, M., 2017. Determination of water quality and effect of aquaculture facilities in bafa lake from Büyük Menderes basin of Turkey. *Fresenius Environ. Bull.*, **26**: 1538-1547.
- SGNAULIN, T., de MELLO, G.L., THOMAS, M.C., GARCIA, J.R.E., de Oca, G.A.R.M. AND EMERENCIANO, M.G.C., 2018. Biofloc technology (BFT): An alternative aquaculture system for piracanjuba, *Brycon orbignyanus*? *Aquaculture*, **485**: 119-123. DOI: 10.1016/j.aquaculture.2017.11.043.
- TOKSEN, E., 2006. Occurrence of gill parasite *Lamellodiscus ignoratus* (Palombi, 1943) (Monogenea : *Diplectanidae*) in cultured sharp-snout sea bream, *Diplodus puntazzo* (Cetti, 1777) from Izmir, Turkey. *Bull. Eur. Assoc. Fish Pathol.*, **26**: 174-179.
- TOKSEN, E. AND CILLI, 2010. Occurrence of parasites in cultured common dentex (*Dentex dentex* L.) from Izmir, Turkey. *Bull. Eur. Assoc. Fish Pathol.*, **30**: 92-98.
- Turkmen, G., 2007. Experimental commercial growout of *Penaeus semisulcatus* (Decapoda : *penaeidae*). *Israeli J. Aquacul-Bamidgeh*, **59**: 52-57.
- TURKMEN, S., CASTRO, P.L., CABALLERO, M.J., HERNANDEZ-CRUZ, C.M., SALEH, R., ZAMORANO, M.J., REGIDOR, J. AND IZQUIERDO, M., 2017. Nutritional stimuli of gilthead seabream (*Sparus aurata*) larvae by dietary fatty acids: effects on larval performance, gene expression and neurogenesis. *Aquacul. Res.*, **48**: 202-213 DOI: 10.1111/are.12874.
- VINATEA, L., MALPARTIDA, J., CARBO, R., ANDREE, KB., GISBERT, E. AND ESTEVEZ, A., 2018. A comparison of recirculation aquaculture systems versus biofloc technology culture system for on-growing of fry of *Tinca tinca* (Cyprinidae) and fry of grey *Mugil cephalus* (Mugilidae) *Aquaculture*, **482**: 155-161. DOI: 10.1016/j.aquaculture.2017.09.041.
- WAITE, R., BEVERIDGE, M., BRUMMETT, R., CASTINE, S., CHAIYAWANNAKARN, N., KAUSHIK, S., MUNGKUNG, R., NAWAPAKPILAI, S. AND PHILLIPS, M., 2014. *Improving productivity and environmental performance of aquaculture*. Working Paper, Installment 5 of Creating a Sustainable Food Future. World Resources Institute. Washington, DC: Accessible at <http://www.worldresourcesreport.org>.
- YANIK, T. AND ARAS, S., 1999. Economical analysis of replacing slaughterhouse byproduct meals in salmonid *Oncorhynchus mykiss* fry diets, *SCI-Expanded. Turkish J. Vet. Anim. Sci.*, **23**:155, 160.

- YIGIT, N. O., DULLUC, A., KOCA, S.B. AND DIDINEN, B.I., 2013. Effects of canola meal use instead of soybean meal in mirror carp (*Cyprinus carpio*, L. 1758) diet on growth and body composition. *J. agric. Sci.*, **19**: 140-147.
- YUCEL-GIER, G., USLU, O. AND KUCUKSEZGIN, F., 2009. Regulating and monitoring marine finfish aquaculture in Turkey. *J. appl. Ichthyol.*, **25**: 686-694 DOI: 10.1111/j.1439-0426.2009.01367.x.

DEVELOPMENT OF AQUACULTURE AND AQUARIUM SECTOR IN TURKEY

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Abstract.- The world population is estimated to be 10 billion in 2050. In this regard, one of the most important of the problems of the world is undoubtedly nourished. As an optimistic development in this regard; aquaculture appearance at a significant level compared to the agricultural sector in many countries. In archaeological investigations found some evidence of the aquaculture being made by the Egyptians for the first time in 2500 BC. Extensive marine farms of seabass, sea bream, mullet, and oyster were produced for the first time in Rome in the 6th century BC. Salmon is the first type of fish that is cultivated in cold waters. Modern aquaculture began about 40 years ago. Many Mediterranean countries took part in this development. Production of aquatic animals from aquaculture in 2015 amounted to 76.6 million tons. Major producers were China (60%), India, Vietnam, Bangladesh, and Egypt. In Turkey, aquaculture began in 1968 with the culture of inland water fish in the terrestrial environment. When examining the data year 2016 concerning the date reached; Turkey's trout production is 101000 tons and carp production has reached 196 tons. The production of marine fishes started in 1984. Aquaculture of marine fishes (151.794 ton in 2016) has entered into a rapid development process in the culture with sea bream (58.254 ton) and sea bass (80.847 ton) fishes, especially on the Aegean coast. All these economic fishes are valuing as a food source. The other fish sector is ornamental fishes and became very popular in all around the world. Most of the ornamental fish lovers usually keep them for recreational purpose or show their rich and high status as a symbol. The global import value for ornamental fish rose from 247.9 million \$ in 2000 to 299 million \$ in 2014. This sector is a multi-million dollar market with considerable growth in the last two decades. USA, Europe, and Japan are the largest markets for aquarium fish. Ornamental fishes come from Asia (65%) to this sector. Aquariums are kept in most shopping centers, cinema and conference halls in Turkey now. Unfortunately, in Turkey, there are not native fish species that are considered ornamental fish species. Most of the ornamental fishes are exotic species and are imported from a foreign country. We can say that as a result of this review; The cultured fish and the ornamental fish sector are the global components of international trade and development and Turkey has taken place in this sector.

Keywords: Aquaponics, aquaculture, Turkey

INTRODUCTION

The population increased about six-fold between the year 1800 and 2000. An average of 140000 children come to the world every day. The estimated population is at around 10 billion in 2050. So, "Where will we find the need for increased protein availability as the result of incredible population growth acceleration and increasing drought over the next 50 years?" There is a large

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portion of this food that must come from farmed protein sources, which may yield extensive growth in the food industry. Unfortunately, due to expanding urbanization and pollution, the world will need to produce this food on a decreasing amount of land suitable for agriculture. In this case, we need to find some new food source or develop other old fashion food sources such as fish. Fish is a quick-to-use and easy-to-prepare product. A good alternative to reducing protein expression is animal protein source and omega-3 (essential unsaturated fatty acid). This animal is very valuable protein source and has a lot of eggs; when they grow up they can serve a potential food for human.

Fish consumption has undergone major changes in the past four decades. World apparent per capita fish consumption has been increasing steadily, from an average of 10 kg in the 1960s to 12 kg in the 1970s, 13 kg in the 1980s, 14 kg in the 1990s and reaching 20 kg in 2015 (Cai and Leung, 2017). There are large variations across countries and regions of the world in the amount of total fish supply for human consumption, reflecting different eating habits and traditions, availability of fish and other foods, prices, socio-economic levels, and seasons (Suzer, 2017). It is good news that improvements in packaging and more efficient and reliable transport can send to all around the world for both originating caught and cultured fish. They usually prefer fresh (45%) and frozen fish (31%).

The most important difference between fish farming obtained by hunting is the lack of feed costs. However, the amount of hunting production has gradually decreased in recent years. After World War II World fisheries production increased rapidly. It is predicted in 2050 that the amount of aquaculture will increase by two and a half times than the fishing.

Aquaculture production is playing an increasing role in satisfying demand for human consumption of fish and fishery products. In the past few years, major increases in the quantity of fish consumed have originated from aquaculture. Despite the increase in the amount obtained through aquaculture, the quantities obtained from fisheries stocks are not expected to increase in the coming years. The total production of fishery and aquaculture in the world was 170 million tons in 2015 (TUIK, 2018). The amount of this production obtained by aquaculture is 77 million tons. In this context, the importance of the amount obtained from the aquaculture emerges.

Turkey's aquatic products are carried out through hunting and farming fish. It has different species of seas from north to south that is increasing

temperature and salinity. In addition, it has 200 natural lakes, more than 300 dam reservoirs, 33 large rivers, and 750 lakes form in its inland waters. These waters constitute its important resources in terms of production of aquatic products. Anchovy (*Engraulis encrasicolus*), horse mackerel (*Trachurus trachurus*), sardine (*Sardina pilchardus*), mackerel (*Scomber scombrus*), bonito (*Sarda sarda*), whiting (*Merlangius merlangus*), bluefish (*Pomatomus saltatrix*), sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*) and trout (*Onchorynchus mykiss*) are the most selling fish species in Turkey (Emiroglu *et al.*, 2017). Aquaculture is most concentrated in seas, dam lakes, natural lakes and cages in some large rivers in Turkey. In addition to cages, concrete and soil ponds are also using for cultivating various aquatic species.

AQUACULTURE IN TURKEY

Major developments have been recorded in our country in recent years in our aquaculture systems. Modern and advanced technologies have started to be used especially in medium and large scale enterprises. In the sea, fish farms transported to open and deep waters and new techniques suitable for these waters have been used. Advanced technology is being used in network cage sizes, structures, and mooring systems. Logistic support has been provided by the introduction of Barge systems and automatic feeding units. Protection and digital tracking systems have been established. The subcontracted industry has been developed and advanced steps have been taken in terms of infrastructure and techniques (Ozden *et al.*, 2017). Depending on these developments in Aquaculture; Export products of 99% to EU countries from Turkey is consist of aquaculture products. It is exported about 115,000 ton of 134 fisheries products to 85 countries (Fig. 1). 68% of the exporting aquatic products have been made to European Union countries in the last five year. Apart from the EU countries; when adding Japan, Russia, and the United States which are the most exported countries, has been grossed to 80 % (TUIK, 2017).

The aquatic products amount in Turkey reached approximately 238 thousand ton today. The average rate of increase in annual production is about 8%, but the rate of increase exceeds 13% in some periods in Turkey. The highest production increase was in trout (130%) followed by sea bream (100%) and perch (52%) (Sariozkan, 2016).

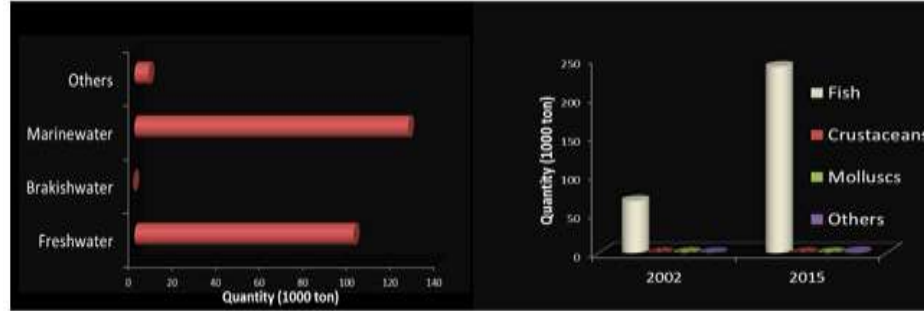


Fig. 1. Aquaculture of Turkey increased 2.2% in 2015 (TUIK, 2017)

Freshwater fishes

Aquaculture activities in Turkey are quite new compared to other countries. In 1969, inland water fish farming has been started by a commercial establishment. They farmed rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) in some artesian wells constructions (Celikkale *et al.*, 1999). After a short time, the same company had set up the first commercial management of the family type by bringing rainbow trout eggs from Austria.

The beginning of the 1990s, another company signed within the framework of a protocol with İstanbul University for producing Atlantic salmon which transferred eyed stage (*Salmo salar*) eggs from Norway. After that, the eggs were subjected to incubation in the hatchery and the obtained juvenile fishes reached a small length in a very short time. Atlantic salmon fry has been sent to businesses established in the Black Sea these years, but salmon production has been abandoned in the following years due to the negativities encountered in raising salmon fishes in the Black Sea (such as water temperature, lack of proper shelter, etc.). Production studies in the cultural environment of the Russian sturgeon (*Acipenser gueldenstaedtii*) in Turkey are still new. 200,000 Russian sturgeon eggs brought from Russia in 2001 were opened in the incubators prepared at the unit and 45.000 fries were left at the end of the same year at the river (Memis *et al.*, 2009).

Turkey is divided into seven geographic regions. There is a total of 1881 freshwater fish farms in Turkey (TUIK, 2017). A large part of these facilities is located in the Aegean Region. The Aegean Region accounts for about 55% of the total production due to the geographical features it has. This region is the first

fish farming in terms of both trout and sea fish (Fig. 2). Approximately 25% of Turkey's production of trout are derived from this region. The Aegean Region is followed by Marmara, the Black Sea, and Mediterranean regions. In Turkey, the number of facilities with a capacity of 1000 tons and over has approached 123 (Fig. 3). Today, trout production (marine growth) in the sea is carried out together with sea bass production in the facilities of Black Sea Region.



Fig. 2. A view of some rainbow trout (*Oncorhynchus mykiss*) farms in Turkey.



Fig. 3. Important freshwater fish species and their quantity (x 1000 ton/year) in Turkey's aquaculture (TUIK, 2017).

Marine fishes

Studies on marine fish culture were increased after the 1980s in Turkey. This situation was parallel with aquaculture developments in, especially Mediterranean countries. The first marine fish hatchery was established at West coast part in İzmir. At the same time, larviculture experiments on European sea

bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) were conducted in Ege University, Faculty of Fisheries. Cultivation of marine fish in Turkey was realized in the first marine fish hatchery established. After that date, the number of larvae production facilities has increased rapidly (Fig. 4). At the 1990s year; shrimp culture studies gained momentum in commercial companies with university cooperation, but no positive results were obtained from these investments.



Fig. 4. Modern marine aquaculture facilities in Turkey. a, marine aquaculture tassis; b, larva culture in tanks; c, net cages (Yildirim, 2004).

The biggest re-organization has occurred after the 2008 economic crisis because little-scale marine hatcheries were sold out of Greek enterprises due to great unemployment and dramatically reducing of commercial gain in the aquaculture sector. The decrease in the number of facilities did not cause a decrease in the number of the breed. Only small companies that can not renew themselves technologically have gotten out of there, and companies that exist in the system have regulated their production capacities, technologies, and marketing systems. End of 2017, there are a total of 22 hatcheries operating in different companies and subsidiary facilities connected to these hatcheries. One of the subsidiary facilities operates only as parental management and the other as a growing facility. There are a total of 427 marine fish farms in Turkey and have

a large-scale marine fish cage in the Mediterranean and the Black Sea Regions (TUIK, 2017).

The European aquaculture, especially the Mediterranean marine aquaculture, has progressed very rapidly over the past 30 years. The countries which are located in the Mediterranean and who are successful in producing know-how, have taken a very important step in the breeding of marine fish larvae. The information produced is shifted to the economic system and from there to the developing countries, the sector growth is ensured. For the first time, marine fish farming studies in France and Italy have been started. It continues today under the leadership of Greece and Turkey. These two countries have almost undertaken the production of marine fishes (sea bream and sea bass) of Europe. Gilted seabream and European Seabass are commonly cultured species in Turkey (Fig. 5). About 200 million sea bass and 180 million sea bream juveniles have been cultured in Turkey today.

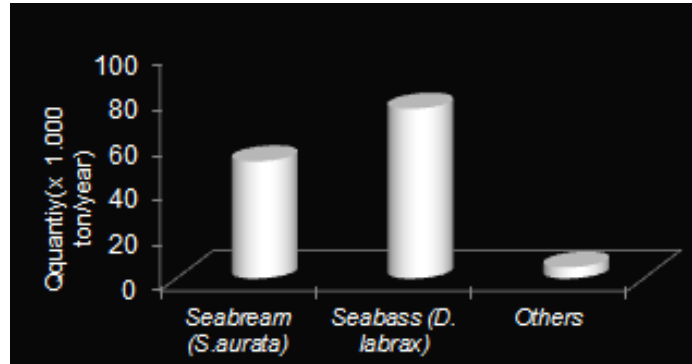


Fig. 5. Important marine water fish species in Turkey's aquaculture (TUIK, 2017).

In recent years, economic crises, especially due to exports, and reduced economic output as a result of increased production of sea bream fish have pushed the search for new species. Approximately 9 fish species have been studied in research studies in Turkey. These candidate fish species are generally grouped in the form of relatively fast-growing fishes (such as Meagre) and slow-growing high-priced fish (such as red porgy). Some companies have been designing the breeding of alternative marine fish species outside sea bream and seabass. In the last 10 years, it has been observed that common dentex, red porgy, turbot, meager, sharp snout sea bream, Atlantic bluefin tuna, shi drum, white seabream, white grouper, leer fish, sand steenbras and redbanded seabream

species are under investigation. In most of these species, very good results have been obtained, but especially in species such as meager, common dentex, and sharp snout seabream very high success has been achieved. In particular, the breeding, larval breeding and growing techniques of Sparidae family species have shown similarities to the production of sea bream, which has been a factor in obtaining good results in research and production studies. Pilot studies have gained importance in the production of species such as white grouper, greater amberjack, and tuna.

The Invertebrates

Mussel farming is a new topic for Turkey's aquaculture industry. It began in a commercial firm (Pinar Company) in 2004. Republic of Turkey Ministry of Agriculture and Rural Affairs arranges some legislations and guidelines for safety and sustainable bivalve production in Turkey. 32 regions are monitored regularly for bivalve production. There are 12 commercial companies with a depuration plant that export shellfish. The amount of farming mussel (*Mytilus galloprovincialis*) is estimated to be around 2 thousand tons per year. Recently, several enterprises have started to culture mussel and also some official permission has been completed for the production especially along the coast of the Marmara Sea. Clam and oyster cultures are expected to be accelerated in Turkey (Serdar, 2016). If the production is started in the mussel farms where the other commercial companies in the evaluation process in the Ministry will establish in the region, mussels will be produced in our country close to 35 thousand tons per year (Fig. 6).

AQUARIUM SECTOR IN TURKEY

The aquarium industry in the world has become a very wide and diverse global industry with more than 2,500 freshwater fish species, about 1,450 sea fish species and more than 650 reefs and other invertebrate marine organisms (Dey, 2016). Most aquarium fish come from tropical and subtropical climates region. The sector is growing around 14% worldwide every year. About 30 freshwater fish species have control over the aquarium sector, such as livebearers, neon tetra, angelfish, goldfish, zebra danio, and discus. While almost 90% of freshwater fish are supplied by aquaculture, the remaining amount is obtained by catching methods. In marine aquariums, the situation is almost exactly the opposite, with about 98% collected from the wild (Dey, 2016).



Fig. 6. According to Yildirim (2004), Turkey is the most suitable method for the long-line method. In this method, the lines formed using ropes and buoys are fixed to the seafloor. Mussel cultivation is done with the help of vertical rope and filo.

Turkey aquarium fish imports are mostly in Singapore, Taiwan, Hong Kong, Thailand, it is made from the Far East Asian countries such as China. It is observed that the Mediterranean and Aegean regions have potential in terms of cultivating aquarium species and meeting the demand for the domestic market due to their tropical climatic characteristics in terms of their climatic conditions (Fig. 7). There are, however, several shortcomings in the scientific literature that show the current state of the industry, such as the number of aquarium businesses, the types of fish served, the sales ratios, and the number of personnel employed.



Fig. 7. Ornamental fish breeding farms private company #17, Their capacity are 10 000-3 000 000 fry/year in 7 cities (Adana, Antalya, Hatay, İzmir, Manisa, Mersin, Ordu) of Turkey.

Turkey is provided within the boundaries of their aquarium breeding facilities although the existing products in the market would have a large part by imports. Considering the imports (Fig. 8) and exports (Figs. 9, 10) of aquarium fish in our country in 2013, it is determined that there are huge differences between imports and exports. It is seen that the aquarium lives of 1 million \$ are imported. When the exports are examined, it is understood that the total value is 48,000. Turkey's imports aquarium fish parallel to the developments in the aquarium industry seems to increase every year after 2001.



Fig. 8. Eskisehir Aquarium. More than 30 thematic aquaria 19-meter aquarium tunnel, 6 terrariums, in which poisonous and tropical amphibians in a 2400m² indoor area. With an interactive design and newest technology the aquariums are worth a visit in Turkey.



Fig. 9. Tunnel Aquarium in Ankara City. One of Turkey's and Europe's 3 largest tunnel aquarium is with a total capacity of 4.5 million liters of waters and 98 meters in length in Ankara city. There are also a restaurant in which lunch or dinner can take in some interesting exhibit on one side, cafes, cinema and gift shops with special products only found at the aquarium.

Although the aquarium industry in Turkey has great importance in the commercial sense, imports constitute the main elements of the trade. The fish imported from abroad are at risk due to carrier diseases due to the lack of necessary health checks. One of the problems that aquarium operators suffer from is the existence of businesses called 'under the stairs' that do not have any official registration or tax sign. It is stated that those who do this business mostly sell on the internet and almost all of these sales are made informal. This situation causes the aquarium operators to lower the sales potential in the sector, and the people who are interested in the aquarium hobby cause this hobby to cool down due to the lack of information. There is a problem in establishing a reliable database related to the sector. The current size of the sector cannot be determined precisely for this reason. This problem is hampering the development of the industry.



Fig. 10. Istanbul Aquarium in Istanbul City. The path continues with cave fish, world rivers, jellyfish, Turkey fish, predatory fish, sharks. Sometimes there are special activities held, such as diving with sharks or Show's fish feeding times.

FISH FEED SECTOR

In Turkey, the fish feed industry has developed in parallel with aquaculture activities. The first fish feed has produced in 1977. Today, there are 22 fish feed production facilities in Turkey, and 70% of these facilities are comprised of facilities that produce 30.000 tons and below per year. As a result of the survey conducted with mentioned facilities, it is found that their annual production amount is between 10.000 and 30.000 tons, that 56% of them produce only fish feed (Emiroglu, 2017).

Among the Mediterranean countries, our country ranks first in fish feed production. These feed factories are more in Western Anatolia. The most

produced feeds are for trout, sea bream, and sea bass. All of the factories that produce fish food are privately-owned and most of these are integrated features such as aquaculture, processing, packaging, import, and export. 5% of the supplied raw materials are organic certified (Ozden, 2017).

CONCLUSIONS

In general, the growth rate of marine fish farming in our country is higher than the annual growth rate of many industries. Nevertheless, the capacity utilization rate of the Aquaculture facilities is 49.8% (Sariozkan, 2016). A low capacity utilization rate is a factor that increases production costs. Also, the use of water areas, production licensing, insurance, mortgage, livestock, credit, and marketing are the most important issues that the industry is currently facing. It is thought that solving these problems within the scope of scientific data and legal. Good governance of aquaculture is a necessary condition for the sector to fully realize its potential for growth. Good governance will also ensure order and sustainability of this growth. Scientific researches can help us to understand how a broad set of variables and indicators influence the farmed fish from fry fish to consumption. Besides, also help to increase operational efficiency and growth.

REFERENCES

- CAI, J. AND LEUNG, P.S, 2017. Short-term projection of global fish demand and supply gaps. *FAO Fish. Aquacul. Tech. Pap. No. 607*. Rome
- DEY, V.K., 2016. The global trade in ornamental fish. *INFOFISH Int.* 4/2016 .
- EMIROGLU, D., TOLON, T., GÜNAY, D. AND ÖZDEN, O., 2017. Condition assessment of high volume fish cases in Turkey. *Turkish J. agric. Econ.* 23:1,p:111-121. ISSN 1303-0183
- FAO, 2016. *The state of world fisheries and aquaculture 2016contributing to food security and nutrition for all*. Rome. p. 200.
- ISGOREN EMIROGLU, D. AND YAPICI, S.N., 2017. *The development of fish feed industry in Turkey*. 1st International Symposium on Limnology and Freshwater Fisheries, limnofish_Abstract_Book, Abstract ID: FPT-181.
- MEMİŞ, D., ERCAN, E., ÇELİKKALE, M.S., TIMUR, M. AND ZARKUA, Z., 2009. Growth and Survival Rate of Russian sturgeon (*A. gueldenstaedtii*) larvae from fertilized eggs to artificial feeding. *Turkish J. Fish. aquat. Sci.* **9**: 47-52.
- OZDEN, O., SUZER, C. AND KORKUT, A.Y., 2017. *Aquaculture in Izmir*. Izmir Balıkçılığı, First Edit, p. 197.
- SARIOZKAN, 2016. Fishing Industry and Economics in Turkey (in Turkish; Türkiye’de Balıkçılık Sektörü ve Ekonomisi). *Turkish J. aquat. Sci.* **31**: 15-22.
- SERDAR, S., 2016. Shellfish production and new approaches in Turkey. *Bull. Japan Fish. Res. Educat. Agen.* 42. p. 90.

- SUZER, C., KORKUT, A.Y. AND OZDEN, O., 2017. *Why should we consume farm fish?* Izmir Balıkçılığı, First Edit, p. 209.
- TUIK, 2018. *Water Products Statistics*, February 2018. <http://www.tarim.gov.tr/>.
- YILDIRIM, Ş., 2004. A Preliminary Study on the Retention of Mediterranean Mussels to Different Materials in Mersin Bay (Urla-İzmir) (in Turkish; Ag Kafeslerde Balık Yetistirciligi Yapılan Mersin Korgezi'nde (Urla-İzmir) Akdeniz Midyesi'nin Degişik Materyallere Tutunma Oranının Araştırılması üzerine Bir ön çalışma. *E.U. J. Fish. Aquat. Sci*, **21**: 249–251.
- ZHOU, X, 2017. An overview of recently published global aquaculture statistics. *FAO Aquacul. Newsl.* 56, March 2017.

**EFFECT OF SOME BIOFERTILIZERS, PLANT NUTRIENTS AND A
BIOCIDE FOR MANAGEMENT OF RENIFORM NEMATODE,
ROTYLENCHULUS RENIFORMIS INFECTING
SUNFLOWER IN EGYPT**

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Abstract.- The reniform nematode, *Rotylenchulus reniformis* attacks a wide range of crops including sunflower, *Helianthus annuus* in Egypt as well as in many parts of the world. Elimination of the nematodes has received attention to minimize damage to plants. Thus, the present study reports the probable effects of some biofertilizers, plant nutrients and a biocide on the development of *R. reniformis* in sunflower and growth of the plant. Three Egyptian bio-fertilizers (BF), i.e. Nitroben, Rizobactrein and Blue-green; and three Egyptian plant nutrients (PN) i.e. Citrein, Kotangein and Kapronite as well as the biocide Nemaless were evaluated at three rates (a lower rate, the recommend rate and a higher rate) for control of *R. reniformis* and improvement of sunflower cv. Giza 101 under greenhouse conditions $30\pm5^{\circ}\text{C}$. All the evaluated compounds significantly reduced ($P\leq 0.05$ and/ or 0.01) the number of juveniles in soil, swollen females and egg-laying females on roots. The reduction varied greatly according to the type of experimented products and rate of application. The highest reduction in the nematode populations, swollen females and egg-laying females was attained with seed coating by Rizobactrein followed by Nitroben as bio-fertilizers while, the least reductions were obtained by using Blue-green as alga biofertilizer followed by Nemaless as a biocide. Application of the plant nutrients, Kotangein as seed coating and Kapronite as soil amendment were effectively decreased the development of the nematode stages. Citrein as a foliar spray nutrient was the least effective. Generally, Rizobactrein and Nitroben as biofertilizers; Kapronite and Kotangein as plant nutrients proved to be the most effective for controlling *R. reniformis* and gave the greatest growth of sunflower plants as compared with the rest treatments.

Key words: Biocides, nematode biocontrol, *Rotylenchulus reniformis*, plant nutrients, sunflower.

INTRODUCTION

Over-reliance on the use of synthetic pesticides in crop protection has resulted in disturbances to the environment, pest resurgence, pest resistance to pesticides and lethal and sub-lethal effects on non-target organisms, including humans (Prakash and Rao, 1997). These side effects have raised public concern about the routine use and safety of chemical nematicides. Also, increases in the populations of plant-parasitic nematodes and a tendency to use ever greater

quantities of pesticides are causing ever greater environmental problems, that is, provision of sufficient clean food whilst at the same time protecting water supplies and wild life habitats. In Egypt, plant parasitic nematodes, especially reniform nematode, *Rotylenchulus reniformis* are important pests and cause considerable loss to many economic crops including sunflower (Oteifa, 1987; Johnson and Fassuliutis, 1984). Therefore, management of this nematode has received attention to reduce damage by encourage scientists to search for synthetic pesticides alternatives. Osman et al. (2005) and Ismail and Hasabo (2000) found that Nemaless (a water suspension of *Serratia marcescens* Bizio containing 1×10^9 bacterium cells / ml water, produced by the Egyptian Ministry of Agriculture and Land Reclamation) reduced the different stages of *Meloidogyne incognita*. Also, these bacterium cells used as potential biocontrol agents against different parasitic nematodes (Mercer et al., 1992; Abd-Elgawad and Mohamed, 2006; Kassab et al., 2017). Also, use of selected marine algae as biocidal agents offers a potential approach to suppress the nematode pests of agricultural crops (Surindar et al., 1987; Ismail and Hasabo, 2000; Youssef and Eissa, 2014). The interrelationships between nematode populations, nutrient elements and plant growth have been reported by Kirkpatrick et al. (1964) and Ismail and Hasabo (2000). Using of powdered sulphur on soil of garlic field caused significant reductions in *Tylenchorhynchus* spp. and *R. reniformis* populations (Kassab and Hafez, 1990). With respect to the relation between trace elements and nematode populations and pathogenicity studies, many reports have been done. Van Gundy and Martin (1961) reported that copper in the leaves of sweet orange seedlings was reduced by *Tylenchulus semipenetrans*. Ashoub (1978) found that eggplants provided with iron (Fe) nutrient solution harboured less number of *R. reniformis* than that of non-treated plants as well as *R. reniformis* was less in cases of Fe or Zn deficiency but not with Mo deficiency (Aboul-Eid et al., 1980). Also, El – Gindi et al. (2005) found no significant differences between nitroben and phosphorine as biofertilizers in reducing *M. incognita* population and cowpea growth responses, however nitroben seems to be more effective than phosphorine on the above mentioned parameters. Therefore, the present study reports the probable effects of some biofertilizers, plant nutrients and a biocide on the development of *R. reniformis* in sunflower, *Helianthus annuus* and growth of the plant in Egypt.

MATERIALS AND METHODS

Two Egyptian biofertilizers i.e. Nitroben and Rizobactrein containing the nitrogen fixing bacteria and one plant nutrient such as Kotangein containing a

mixture of microelements (Fe, Zn and Mn) with sulphur were used as seed coating to sunflower seeds. Other treatments included foliar application with one plant nutrient i.e. Sitrein (mixture of 6% Fe, Mn and Zn with 15% citric acid). Blue- green algae as biofertilizer; Kapronite as plant nutrient containing a mixture of elemental sulphur and substances enriched with P, K, Ca, Mg and Nemaless as a biocide containing a strain of bacteria *Serratia marcescens* were used as soil treatment. All the previous biofertilizers, plant nutrients and a biocide were applied at the recommended rate as well as half and double rates. Seeds of sunflower, *Helianthus annuus* cv. Giza 101 were sown in 20 cm diameter clay pots filled with 2 kg autoclaved soil mixture of sand and clay (1:1, v:v). After germination, only one healthy plant was kept in each pot. After 15 days of germination, six replicates were prepared from each treatment and each plant was inoculated with 1000 freshly hatched *Rotylenchulus reniformis* fourth stage juveniles (J₄). Untreated pots inoculated with nematodes served as control. Pots were arranged in a randomized complete block design in a greenhouse at 30± 5°C. Soil treatments were applied at sowing time; foliar spray nutrients were applied twice (at 3 weeks old plants then 3 weeks later). Seed coating treatments were made by mixing the seeds in Arab gum and the tested material for 2-5 minutes and then left to dry for 4 hours before sowing. Seventy days after nematode inoculation, sunflower plants were gently uprooted and the fourth-stage juveniles (J₄) in the soil were extracted by sieving and centrifugation (Barker et al., 1986). Number of swollen females and egg-laying females were counted for the whole root system. Lengths, fresh and dry weights of both shoot and root systems were recorded. The percentages reduction or increase in the nematode population or plant growth parameters as compared to untreated plants were calculated. Data were analyzed statistically using the Fisher's Least Significant Difference (L.S.D.).

RESULTS

Tables I and II showed that using of the biofertilizers (BF), the plant nutrients (PN) and the biocide at the three rates significantly ($P \leq 0.05$ and / or 0.01 levels) reduced numbers of juveniles in soil, swollen females and egg-laying females on roots as compared to untreated plants. In the previous nematode stages, statistical differences at 0.05 and / or 0.01 levels were noted within some treatments. The reduction greatly varied according to the type of the evaluated materials. So, the highest decrease in numbers of juveniles in soil, swollen females and egg-laying females was more clearer with seed coating by Rizobactrein followed by Nitrobien biofertilizers (Table I). However, the least

decreases in the nematode stages were obtained when plants treated with Blue green algae biofertilizer followed by Nemaless biocide. With respect to the impact of the tested plant and soil nutrients (Table II), application of Kotangein as seed coating and Kapronite as soil amendment showed highest percentage reduction; respectively in the numbers of juveniles in soil, swollen females and egg-laying females followed by Citrein treatment.

TABLE I.- EFFECT OF SOME BIO-FERTILIZERS AND A BIOCIDES ON THE DEVELOPMENT OF ROTYLENCHULUS RENIFORMIS. *(MEAN OF TWO SUCCESSIVE SEASONS, 2015 & 2016).

Treatment and type of application	Dose	No. of juveniles in soil	Reduction (%)	No. of swollen females / root	Reduction (%)	No. of egg-laying females / root	Reduction (%)
Nitrogenous bio-fertilizers (g / g of seeds)							
Nitroben (seed coating)	0.034	2150	50.9	160	36.0	110	24.1
	0.068	1900	56.6	145	42.0	95	34.5
	0.136	1370	68.7	133	46.8	81	44.1
Rizobactrein (seed coating)	0.017	1395	68.2	110	56.0	88	39.3
	0.034	1100	74.9	98	60.8	75	48.3
	0.068	630	85.6	78	68.8	51	64.8
Blue-green Algae (g / pot)							
Blue – green (soil)	0.2	3900	11.0	233	6.8	140	3.5
	0.4	3760	14.2	215	14.0	131	9.7
	0.8	3250	25.8	210	16.0	129	11.0
Biocide (ml / pot)							
Nemaless (soil)	0.005	3050	30.4	212	15.2	128	11.7
	0.01	2860	34.7	190	24.0	110	24.1
	0.02	2410	45.0	177	29.2	107	26.2
Control	-	4380	-	250	-	145	-
L.S.D. 5%	-	410	-	15	-	17	-
L.S.D. 1%	-	620	-	23	-	31	-

*Data given represent the mean of six replicates.

**Percentage reduction compared to control.

The influence of the various rates of BF, PN and Nemaless on the growth of sunflower plants infected with *R. reniformis* is presented in Tables III and IV. All treatments with the different rates significantly increased plant growth ($P \leq 0.05$)

and / or 0.01 levels), with some exceptions, as compared to untreated plants. Statistical differences at 0.05 and / or 0.01 levels in all shoot and root systems growth parameters were observed within some treatments. However, insignificant variations, with some exceptions, were noted between the tested three rates of each treatment in all plant growth criteria (Tables III and IV). The increase in plant growth parameters varied according to the type of the evaluated materials. Therefore, the highest increase in lengths, fresh and dry weights of both shoot and root systems were obtained by using Rizobactrein followed by Nitroben as biofertilizers (Table III). Using of Kotangein (seed coating) and Kapronite (soil amendment) as plant nutrients showed the highest percentage increase in the plant growth parameters while; the least increases were observed in plants treated with Citrein (Table IV). Clearly, stronger responses were obtained in shoot growth parameters compared to root growth parameters (Tables III, IV).

TABLE II.- EFFECT OF SOME PLANT AND SOIL NUTRIENTS ON THE DEVELOPMENT OF ROTYLENCHULUS RENIFORMIS. *(MEAN OF TWO SUCCESSIVE SEASONS, 2015 & 2016).

Treatment and type of application	Dose	No. of juveniles in soil	Reduction (%)	No. of swollen females / root	Reduction (%)	No. of egg-laying females / root	Reduction (%)
Foliar spray nutrients (ml / plant)							
Citrein	0.1	3430	21.7	215	14.0	136	6.2
	0.2	2950	32.7	195	22.0	125	13.8
	0.4	2710	38.1	183	26.8	110	24.1
Seed coating (g / g of seeds)							
Kotangein	0.01	1210	72.4	153	38.8	110	24.1
	0.02	950	78.3	110	56.0	93	35.9
	0.03	910	79.2	98	60.8	78	46.2
Soil amendment (gm / pot)							
Kapronite	1.0	2100	52.1	198	20.8	122	15.9
	2.0	1830	58.2	173	30.8	107	26.2
	4.0	1100	74.9	161	35.6	98	32.4
Control	-	4380	-	250	-	145	-
L.S.D. 5%	-	1010	-	12	-	14	-
L.S.D. 1%	-	1400	-	19	-	25	-

*Data given represent the mean of six replicates.

**Percentage reduction compared to control.

TABLE III.- EFFECT OF SOME BIO-FERTILIZERS AND A BIOCIDES ON THE GROWTH OF SUNFLOWER INFECTED WITH R. RENIFORMIS. * (MEAN OF TWO SUCCESSIVE SEASONS, 2015 & 2016).

Treatment and type of application	Dose	Shoot growth					Root growth						
		Fresh weight (g)	Increase % **	Dry weight (g)	Increase %	Length (cm)	Increase %	Fresh weight (g)	Increase %	Dry weight (g)	Increase %	Length (cm)	Increase %
Nitrogenous bio-fertilizers (g / g of seeds)													
Nitroben (seed coating)	0.034	7.5	36.4	1.8	100	45.1	17.8	7.6	182	2.0	186	22.6	28.4
	0.068	9.0	63.6	2.3	156	47.3	23.5	8.3	207	2.4	243	24.3	38.1
Rizobactrein (seed coating)	0.136	12.0	118.2	3.1	244	48.9	27.7	9.7	259	2.9	314	26.2	48.9
	0.017	12.0	118.2	2.2	144	46.5	21.4	8.1	200	2.1	200	25.3	43.8
	0.034	13.9	152.7	2.7	200	49.8	30.0	9.3	244	2.6	271	26.1	48.3
	0.068	14.9	170.9	3.9	333	51.3	33.9	11	311	3.1	343	28.3	60.8
Blue-green Algae (gm / pot)													
Blue – green (soil)	0.2	6.0	9.1	1.5	66.7	43.6	13.8	6.5	141	1.5	114	17.8	1.1
	0.4	7.9	43.6	1.9	111	45.9	19.8	7.1	163	1.9	171	20.1	14.2
	0.8	9.3	69.1	2.4	167	48.4	26.4	8.2	204	2.1	200	21.7	23.3
Biocide (ml / pot)													
Nemaless (soil)	0.005	6.5	18.2	1.6	77.8	44.8	17.0	7.1	163	1.9	171	19.7	11.9
	0.01	8.6	56.4	2.1	133	46.7	21.9	7.9	193	2.2	214	21.4	21.6
	0.02	9.9	80.1	2.8	211	49.1	28.2	8.8	226	2.6	271	23.5	33.5
Control	-	5.5	-	0.9	-	38.3	-	2.7	-	0.7	-	17.6	-
L.S.D. 5%	-	3.6	-	0.7	-	6.3	-	4.4	-	0.4	-	4.6	-
L.S.D. 1%	-	5.3	-	1.8	-	8.6	-	6.1	-	0.6	-	6.2	-

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TABLE IV.- EFFECT OF SOME PLANT AND SOIL NUTRIENTS ON THE GROWTH OF SUNFLOWER INFECTED WITH R. RENIFORMIS.* (MEAN OF TWO SUCCESSIVE SEASONS, 2015 & 2016).

Treatment and type of application	Dose	Shoot growth				Root growth							
		Fresh weight (g)	Increase % ***	Dry weight (g)	Increase %	Length (cm)	Increase %	Fresh weight (g)	Increase %	Dry weight (g)	Increase %	Length (cm)	
Foliar spray nutrients (ml / plant)													
Citrein	0.1	9.1	65.5	2.4	167	43.9	15	3.6	33	1.3	86	22.8	29.6
	0.2	10.1	83.6	3.6	300	44.3	16	4.1	52	1.4	100	23.3	32.4
	0.4	10.6	92.7	3.7	311	45.6	19	4.9	82	1.8	157	24.6	39.8
Seed coating (g / g of seeds)													
Kotangenin	0.01	10.8	96.3	3.1	244	47.6	24	4.9	82	1.5	114	24.7	40.3
	0.02	11.3	106	3.9	333	48.1	26	5.3	96	1.8	157	25.2	43.2
	0.03	11.9	116	4.0	344	50.3	31	5.9	119	2.1	200	25.9	47.2
Soil amendment (g / pot)													
Kapronite	1.0	9.9	80.0	2.8	211	45.1	18	4.1	52	1.2	71	23.6	34.1
	2.0	10.2	85.5	3.7	311	46.7	22	5.1	89	1.7	143	24.1	36.9
	4.0	10.9	98.2	3.9	333	48.4	26	5.3	96	1.8	157	25.3	43.8
Control	-	5.5	-	0.9	-	38.3	-	2.7	-	0.7	-	17.6	-
L.S.D. 5%	-	2.9	-	0.6	-	6.3	-	0.9	-	0.4	-	3.2	-
L.S.D. 1%	-	4.1	-	1.7	-	8.2	-	1.1	-	0.6	-	4.6	-

DISCUSSION

The evaluated biofertilizers, plant nutrients and a biocide had a negative effect on the development of *R. reniformis* on sunflower. This was evident by the lower numbers of juveniles in soil, lower numbers of swollen females and egg-laying females and this coincided with improvement in plant growth of the treated pots. These findings are in harmony with those of Featomby – Smith and Van Standen (1983), Kassab and Hafez (1990), Ali and Kamal (1998), Abd-Elgawad and Mohamed (2006) and Kassab et al. (2017). They stated that the application of iron nutrient solution; brown alga; powdered sulphur and diluted liquid culture of the bacteria *Serratia marcescens* significantly suppressed several species of plant-parasitic nematodes and improved the growth of the host crops. Also, El-Sherif et al. (1994), Ali (1996), Ismail and Hasabo(2000), Abd-Elgawad and Mohamed (2006) and Kassab et al. (2017) reported that liquid cultures of *Serratia* sp. (the major component of the biocide Nemaless) or its filtrates inhibited egg hatching and juvenile survival of different plant-parasitic nematodes. The role of these bacteria may be attributed to the accumulation of toxic metabolites of these bioagents in soil. These metabolites may have a direct lethal effect on nematodes (Dicklow et al., 1993), or have some physiological and / or behavioral effects such as disorder of neuromuscular junctions or through suppression of hatching, movement, feeding and invasion to host tissue (Mishra et al., 1987; Kluepfel et al.,1993). For addition, ammonia produced by ammonifying bacteria during natural decomposition of nitrogenous products has been often implicated in the control of plant parasitic nematodes (Rodriguez, 1986). Fatty acids, volatile compounds, hydrogen sulfide, enzymes, hormones, alcohol and phenolic compounds are among the bacterial metabolic products implicated in the management of plant parasitic nematodes (Mishra et al., 1987; Ismail and Hasabo, 2000; Abd-Elgawad and Mohamed, 2006; Kassab et al., 2017). These products may be toxic to nematodes directly or it may indirectly suppress nematode population by modifying the rhizosphere environment.

The increase in plant growth as compared to the untreated control could be attributed to decreasing of the nematode population and to the addition of organic or inorganic compounds contained in the bacterial metabolites. Moreover, most of these products improved the physical and chemical properties of soil and provides the soil with components that help solubilization and absorption of many macro and micro elements by plants which encourage plant growth. The positive benefits from seed coating with Kotangein which contained a mixture of microelements (Fe, Zn and Mn) with sulphur have been attributed increased root

uptake capacity because of enhanced root development and hair formation in response to secretion of plant growth hormones (Owen and Novotny, 1960; Ismail and Hasabo, 2000). Overall, it could be concluded that the net effect of these substances is therefore, combating *R. reniformis* populations and improving sunflower growth through a non-toxic, biological control system which is clearly evident in this study. More studies are, however, in progress to elucidate the action of afore-mention products on other plant-parasitic nematodes attacking various economic crops.

REFERENCES

- ABD-ELGAWAD, M.M. AND MOHAMED, M.M., 2006. Efficacy of selected bio-control agents on *Meloidogyne incognita* on eggplant. *Nematol. Medit.* **34**: 105-109.
- ABOUL-EID, H.Z., OSMAN, H.A. AND ABDEL-FATTAH, A.S. 1980. Effects of *Rotylenchulus reniformis* on pea plants grown under zinc, iron or molybdenum deficiencies. *Afr. J. agric. Sci.* **7**: 13-22.
- ALI, A.H., 1996. Biocontrol of reniform and root-knot nematodes by new bacterial isolates. *Bull. Fac. Agric., Univ. Cairo*, **47**: 487 – 498.
- ALI, A.H. AND KAMAL, H.M., 1998. Biocontrol of root-knot nematode, *Meloidogyne incognita* on table grape. *Bull. Fac. Agric., Univ. Cairo*, **49**: 435 – 452.
- ASHOUB, A.A., 1978. Physio-pathological studies of eggplant, *Solanum melongena* infected with *Rotylenchulus reniformis*. M. Sc. thesis, Fac. Agric. Cairo Univ. pp 113.
- BARKER, K.R., TOWNSHEND, J.L., BIRD, G.W., THOMASON, I.J. AND DICKSON, D.W., 1986. Determining nematode population responses to control agents. In: *Methods for evaluating pesticides for control of plant pathogens* (ed. K.D. Hickey), APS Press, Minnesota.
- DICKLOW, M.B., ACOSTA, N.A. AND ZUCKERMAN, 1993. A novel *Streptomyces* species for controlling plant – parasitic nematodes. *J. Chem. Ecol. USA*, **19**: 159 – 173.
- EL-GINDI, A.Y., OSMAN, H.A., YOUSSEF, M.M.A., AMEEN, H.H., AND LASHEIN, A.M., 2005. Nematicidal effects of some organic amendments, biofertilizers and intercropped marigold, *Tagetes erecta* on the root-knot nematode, *Meloidogyne incognita* infected cowpea plants. *Bull. Egypt. Nat. Res. Cent.*, **30**: 307 – 315.
- EL-SHERIF, M.A., ALI, A.H. AND BARAKAT, M.I., 1994. Suppressive bacteria associated with plant – parasitic nematodes in Egyptian agriculture. *Jap. J. Nematol.*, **24**: 55 – 59.
- FEATOMBY- SMITH, B.C. AND VAN STANDEN, J., 1983. The effect of seaweed concentrate on the growth of tomato plants in nematode-infested soil. *Scient. Horticul.*, **20**: 137 – 146.
- ISMAIL, A.E. AND HASABO, S.A., 2000. Evaluation of some new Egyptian commercial biofertilizers, plant nutrients and a biocide against *Meloidogyne incognita* root knot nematode infecting sunflower. *Pak. J. Nematol.*, **18**: 39-49.
- JOHNSON, A.Y. AND FASSULIUTIS, G., 1984. Nematodes parasites of vegetable crops. In: *Plant and insect nematodes* (ed. W. R. Nickle). Marcel Dekker. New York. pp. 323-372.
- KASSAB, A.S. AND HAFEZ, S.M., 1990. Use of powdered sulfur against the bulb mite,

- Rhizoglyphus robini* and its effect on nematodes in garlic field soil. *Anal. Agric. Sci., Fac. Agric., Ain Shams Univ., Cairo, Egypt*, **35**: 533 – 541.
- KASSAB, S.A., EISSA, M.F.M., BADR, U.M., ISMAIL, A.E., ABDEL-RAZIK, A.B. AND GAZIEA, M.S., 2017. Nematicidal effect of a wild type of *Serratia marcescens* and its mutants against *Meloidogyne incognita* juveniles. *Egypt. J. Agronomatol.*, **16**: 95-114.
- KIRKPATRICK, J.D., MAI, W.F., PARKER, K.G. AND FISHER, E.G., 1964. Effect of phosphorus and potassium nutrition of sour cherry on the soil population levels of five plant-parasitic nematodes. *Phytopathology*, **54**: 706 – 712.
- KLUEPFEL, D.A., MACLENNIA, T.M. AND ZEHAR, E.I., 1993. Involvement of root colonizing bacteria in peach orchard soil suppressive of the nematode, *Criconebella xenoplax*. *Phytopathology*, **76**: 1366 – 1369.
- MERCER, C.F., GREENWOOD, D.R. AND GRANT, J.L., 1992. Effect of plant and microbial chitinases on the eggs and juveniles of *Meloidogyne hapla*. *Nematologica*, **38**: 227-236.
- MISHRA, S.K., KELLER, J.E., MILLER, J.R., HEISEY, R.M., NAIR, M.G. AND PUTNAM, A.R. 1987. Insecticidal and nematicidal properties of microbial metabolites. *J. Indust. Microbiol.*, **2**: 267 – 276.
- OSMAN, H.A., EL-GINDI, A.Y., AMEEN, H.H., YOUSSEF, M.M. AND LASHEIN, A.M., 2005. Evaluation of the nematicidal effects of smashed garlic, sincocin and nemaless on the root-knot nematode, *Meloidogyne incognita* infecting cowpea plants. *Bull. Egypt. Nat. Res. Cent.*, **30**: 297-305.
- OTEIFA, B.A., 1987. Nematode problems of winter season crops in the Mediterranean region. In: *Proceedings of a workshop on parasitic nematodes in temperate semi-arid regions* (eds. Saxens, M.C. and Sikora, J.P.), held at Larnaca Cyprus Mar. 105; Aleppo; ICARDA, pp. 199-207.
- OWEN, R.G. AND NOVOTNY, 1960. Physiological and biochemical studies on nematode galls (Abst.). *Phytopathology*, **50**: 650.
- PRAKASH, A. AND RAO, J., 1997. Botanical pesticides in agriculture. CRC Press, Boca Raton, Florida, USA, pp. 461.
- RODRIGUEZ, K.R., 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *J. Nematol.*, **18**: 129 – 135.
- SURINDAR, P., ARMEN, C.T. AND LYNN, M.H., 1987. Effective use of marine algal products in the management of plant-parasitic nematodes. *J. Nematol.*, **19**: 194-200.
- VAN-GUNDY, S.D. AND MARTIN, J.P., 1961. Influence of *Tylenchulus semipenetrans* on the growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios. *Phytopathology*, **51**: 146 – 151.
- YOUSSEF, M.M.A. AND EISSA, M.F.M., 2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. *J. Biotech. Pharmaceut. Res.*, **5**: 1- 6.

PREVALENCE AND SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACILLI FROM CLINICAL SAMPLES IN ISLAMABAD AND RAWALPINDI

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Abstract.- Gram negative bacterial infections execute massive load on medical structures because of great incidence of community-acquired and nosocomial infections. Occurrence and prompt blowout of resistance producing enzymes in Enterobacteriaceae, Pseudomonas and also in Acinetobacter species is becoming a most important communal healthiness disaster globally, as well as accountable for huge extent of infections. To combat this evolving resistance a constant investigation of antibiotics vulnerability design of gram negative bacteria is vital at local levels that can then afford support in management of operational preliminary treatments. Clinical isolates of gram negative Bacilli were studied in terms of sample origin and patient demographics. The data was statistically analyzed using Chi-square (χ^2) test, confidence interval (CI) and odds ratio analysis for paired samples. The multiple antibiotic resistance index (MARI) of different antibiotics against isolated gram negative bacteria was calculated. A total of 1522 clinical samples were included in this study. Out of which 341 were identified as gram negative bacteria. The overall prevalence of bacterial infections caused by gram negative bacteria was predominantly observed in female patients. Among 341 isolates of gram negative bacteria, 252 belonged to family Enterobacteriaceae whereas 58 were Non-Enterobacteriaceae. A difference in the antibiotic susceptibility profiles of Enterobacteriaceae and Non-Enterobacteriaceae was observed. The predominance of bacterial infections has been found in females and in patients greater than 40 years. CTX, TEC and LEV were found the highest resistant drugs. However, both ERY and TGC were found the most susceptible drugs showed the highest sensitivity.

Keywords: Gram negative bacteria, Pseudomonas, Acinetobacter, antibiotic resistance index

INTRODUCTION

Gram negative bacilli are a diverse group of bacteria that are identified in many infections such as sepsis, pneumonia, urinary tract and postsurgical infections in the hospitals (Foxman and Brown, 2003). They are extensively distributed in the environment and also supported by the intestinal tract of human and most animals. Mostly, they are not disease causing. Escherichia coli are the most frequently isolated gram negative bacilli, which is associated with the urinary and intestinal tract infections. Other disease causing members of

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Enterobacteriaceae include *Serratia marcescens*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes* that are opportunists and are involved in causing nosocomial infections (Peleg and Hooper, 2010). The advancements in antibiotic production ensured the supreme imperative development in the field of medication for treatment against bacterial infections. The word “antibiotic” is a Greek word which means “against life”. Selman Waksman used this word in 1942, to refer any material secreted by a microorganism which is unsuitable for the survival of other microorganism. Normally, this term is defined as a material that either kills or hinders the development of bacteria (Pelczar et al., 1999). Overuse and misuse of antibiotics for the treatment of infections have led to the development of antibiotic resistance among bacterial pathogens. There are many remarkable beneficial gains of these antibiotics but universally they are becoming useless and void with time, due to the resistant strains of bacteria (Yezli et al., 2014). The worldwide scarcity of the new innovative antibacterial drugs and the blowout in the mechanisms of multidrug antibacterial resistance both processes are restraining the doctors in the facility to offer operational and harmless cures for the very vulnerable patients, regrettably. In gram negative bacilli the process of antibacterial resistance is mostly thought provoking. The chief emerging pressure in the antibiotics resistance is the group of renowned pathogens ESKAPE that are; *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas aeruginosa* and *Enterobacter* species. Majority consist on the gram negative bacilli (Boucher et al., 2009). Because of intensifying hazards of antibiotic resistant processes, management of therapeutics in patients having infectious illnesses become problematic.

A systematic checking of the scattering of these bacteria on the basis of sequential differences and on indigenous ranks their antibiotic resistant profiles are essential in the advancement of operative approaches to improve the settings of prone inhabitants. Factors like nature of bacterial infection, preceding record of antibiotic dealings, sexual category, stages of life (age) as well as confined resistant rate have to be accounted to explore the exact comprehensive figures on antibacterial susceptibility (Al’os, 2005). The objectives of the present study were to determine (i) the prevalence of gram negative bacilli isolated from various clinical samples such as blood, breast milk, cerebrospinal fluid, ear swab, pus, semen, sputum, stool, throat swab and urine in Rawalpindi and Islamabad, (ii) the prevalence of Gram negative bacilli isolated from various clinical samples with respect to patients’ gender and age groups in Rawalpindi and Islamabad, and (iii) the antibiotic susceptibility profiles of Gram negative bacilli isolated from various clinical samples.

MATERIALS AND METHODS

Study design

A retrospective study was carried out to ascertain the resistance patterns of clinical isolates of gram negative bacilli in Rawalpindi and Islamabad for a period of six months. Patient samples included blood, breast milk, cerebrospinal fluid (CSF), ear swabs, pus, semen, sputum, stool, throat swab and urine. Sensitivity reports plus cultures were gathered from Microbiology Department of various tertiary care hospitals for study period. The data was analyzed in terms of sample origin relative to patient demographics as well as the respective antibiotic resistance patterns.

Analysis of data

Statistical analysis of the data was performed using Chi square (χ^2) test, Confidence interval (CI), Odds ratio analysis and Student's t-test for paired samples. The relative risks and odds ratio were executed to link the risk factors within dissimilar collections (prevalence of bacterial infections; male and female patients. A P values of <0.05 was considered as statistically significant and at 95 percent level of confidence interval. All statistical tests were performed by GraphPad PRISM software (version 6.0), Inc. 7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA.

Multiple antibiotics resistance index (MARI) for every antibiotic alongside sequestered gram negative bacteria was calculated as:

“MAR index for an antibiotic = no. of antibiotic resistant to isolates / (no. of antibiotics \times no of isolates)”.

The number of MAR index for an antibiotic indicates its sensitivity and resistance. The MAR indices of antibiotics showed their resistance and sensitivity. The antibiotic resistance increases by increase in the value of MAR index (Tambekar et al., 2016).

RESULTS

Overall 1522 clinical samples were studied; significant bacterial growth was observed in 550 (36.14%) clinical samples. The prevalence of various clinical infections caused by bacteria was analyzed by Pearson Chi square test; a

P value of <0.0001 indicated a significant difference between the prevalence rates of different bacterial infections (Fig. 1). As far as gender-wise prevalence of bacterial infections is concerned, a preponderance of female patients over male patients was observed with an overall ratio of 1:1.12 (259 males: 291 females). This gender-wise prevalence of bacterial infections was statistically analyzed by applying Pearson Chi-square test; a P value > 0.05 ($P = 0.91$) indicated a statistically non-significance of the results; risk- and odds ratios > 1 at 95% level of confidence interval showed that males were relatively at a higher risk of contacting any bacterial infection (Table I).

Bacterial infections caused by Gram negative pathogens

Among 1522 clinical samples collected, 1313 (86.27%) were tested for the presence of gram negative bacterial pathogens and a prevalence of 25.97% ($n=341$) was found for infections caused by them. The prevalence of various clinical infections caused by bacterial pathogens was analyzed by Pearson Chi square test; a P value of <0.0001 indicated a significant difference between the prevalence rates of different bacterial infections (Fig. 1).

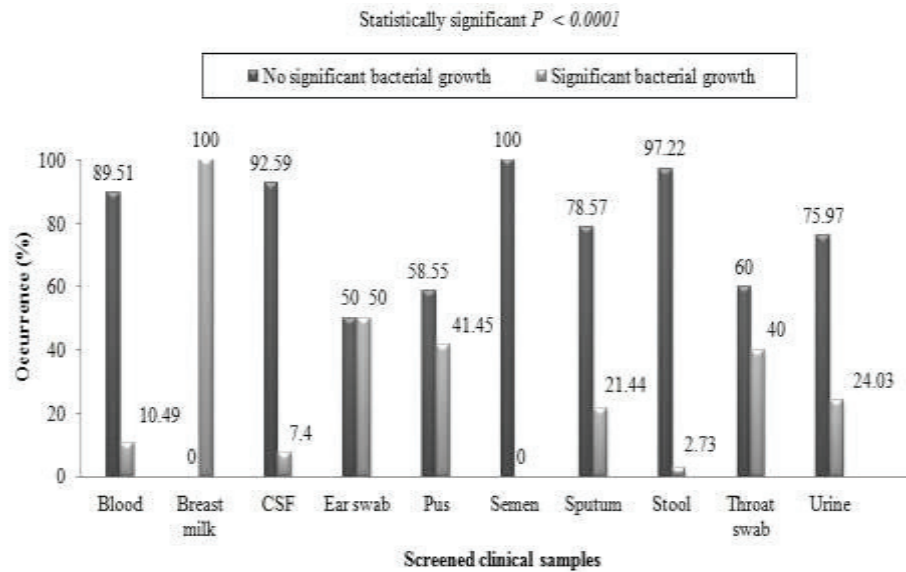


Fig. 1. Distribution of significant and non-significant growth pattern of pathogenic bacteria with respect to clinical samples in Rawalpindi and Islamabad.

TABLE I.- DISTRIBUTION OF SIGNIFICANT AND NON-SIGNIFICANT GROWTH PATTERN OF SCREENED CLINICAL SAMPLES WITH RESPECT TO GENDER IN RAWALPINDI AND ISLAMABAD.

Gender	Samples tested (N)	Clinical Samples				Odds			Risk ratio			χ^2	P value		
		No significant growth	Significant growth	n	%	Odds for each group	95% CI		Risk for each group	95% CI					
							Lower	Upper		Lower	Upper				
Males	683	424	62	259	38	0.61	1.15	0.93	1.41	0.14	0.37	1.09	0.95	1.24	1.71
Females	839	548	65	291	35	0.53					0.34				
Total	1522	972	64	550	36										

TABLE II.- DISTRIBUTION OF SIGNIFICANT AND NON-SIGNIFICANT GROWTH PATTERN OF GRAM NEGATIVE SCREENED CLINICAL SAMPLES WITH RESPECT TO GENDER IN RAWALPINDI AND ISLAMABAD.

Gender	Samples tested (N)	Clinical Samples				Odds		Risk ratio		Pearson Chi-square value	P value					
		No significant growth	Significant growth	N	%	Odds for each group	95% CI		Risk for each group			95% CI				
							Lower	Upper				Lower	Upper			
Males	569	424	74.5	145	25.5	0.34	0.95	0.74	1.22	0.04	0.25	0.96	0.25	0.26	0.72	0.77
Females	744	548	73.7	196	26.3	0.35					0.26					
Total	1313	972	74	341	26											

As far as gender-wise prevalence of infections caused by gram negative bacterial pathogens is concerned, a preponderance of female patients over male patients was observed with an overall ratio of 1:1.35 (145 males:196 females). Among total 1313 clinical samples, 569 (43.34%) were collected from males while 744 (56.66%) from females. Out of 569 clinical samples collected from males, gram negative pathogenic bacteria were isolated from 145 (25.50%). Likewise, out of 744 clinical samples collected from females, gram negative bacterial pathogens were isolated from 196 (26.30%). This gender-wise prevalence of bacterial infections caused by gram negative bacteria was statistically analyzed by applying Pearson Chi-square test; a P value > 0.05 ($P = 0.77$) indicated a statistically non-significance of the results; risk- and odds ratios < 1 at 95% level of confidence interval showed that males were relatively at a lower risk of contacting any bacterial infection caused by gram negative pathogens (Table II). The gender-wise prevalence of various clinical infections caused by gram negative bacterial pathogens was analyzed by Pearson Chi square test; a P value of < 0.05 ($P = 0.0006$) indicated a significant difference between the prevalence rates of different bacterial infections in male and female patients (Fig. 2).

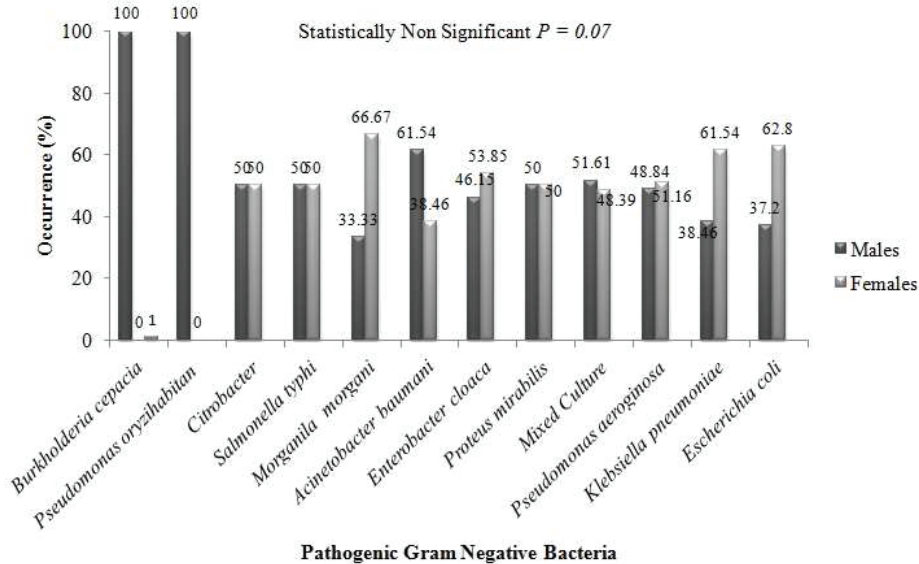


Fig. 2. Gender-wise distribution of pathogenic gram negative bacteria isolated from various clinical samples in Rawalpindi and Islamabad.

As far as age-wise prevalence is concerned, the highest number of bacterial infections was observed in the age group 41-65 years ($n=125$; 36.66%). In males, the highest number of bacterial infections was observed in the age group 41-65 years ($n=56/145$; 38.62%) whereas in females, 20-40 years was the age group with the highest frequency of bacterial infections caused by gram negative bacteria ($n=75/196$; 38.26%). Only female patients were observed in the age group < 1 years ($n=4$). Overall the highest female to male ratio was observed in the age group 41-65 years (1.60:1.00). The Chi-square analysis of the data did not show any significant difference in male and female patients, as indicated by the P value > 0.05 ($P = 0.28$) (Fig. 3).

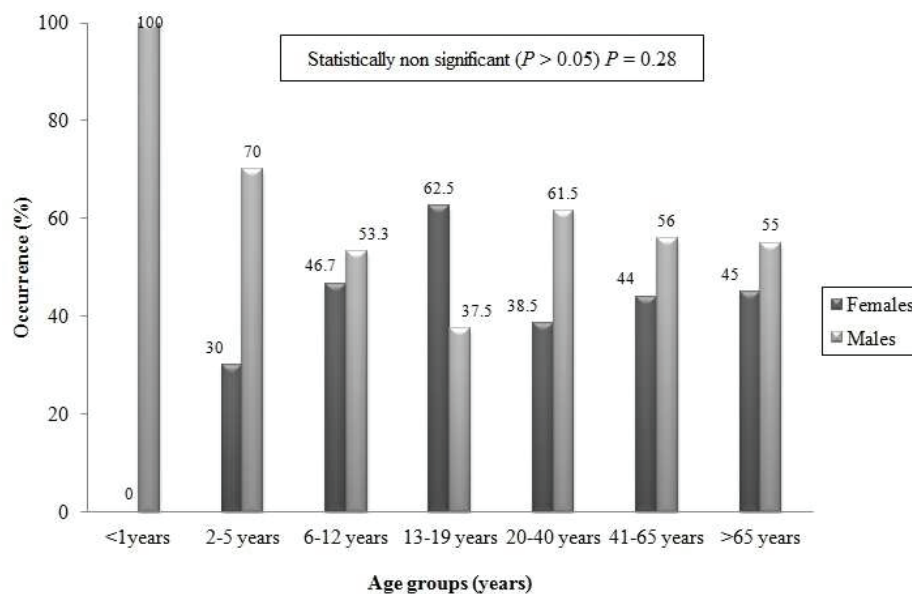


Fig. 3. Age-wise prevalence of bacterial infections caused by gram negative pathogenic bacteria with respect to male and female patients in Rawalpindi and Islamabad.

The distribution of gram negative pathogenic bacteria recovered from various clinical samples was varied. A higher frequency of pathogens belonged to family Enterobacteriaceae as expected. Among a total of 341 total clinical samples with significant bacterial infections, 252 (73.90%) were Enterobacteria while 58 (26.09%) were non-Enterobacteriaceae. Overall, *Escherichia coli* was the most frequently isolated gram negative pathogen ($n=164/341$; 48.09%).

Among Enterobacteriaceae, *E. coli* was the most frequently isolated pathogen ($n=164/252$; 65%) and *Citrobacter* spp. and *Salmonella typhi* were the least frequent ($n=2/252$; 0.79% each); on the other hand, among non-Enterobacteriaceae, *Pseudomonas aeruginosa* was the most frequently isolated pathogen ($n=43/58$; 74.13%) whereas *Burkholderia cepacia* and *Pseudomonas oryzae* were least frequent ($n=1/58$; 1.72% each).

Overall the prevalence rates for the occurrence of gram negative pathogens were predominant in females since on average 59.47% of *M. morganii*, *E. cloacae*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* were isolated from clinical samples obtained from female patients. Nevertheless, the prevalence rates for the occurrence of *B. cepacia* (100%), *P. oryzae* (100%) and *A. baumannii* (61.54%) were higher in male patients. An equal prevalence rate was found among males and females for the occurrence of *P. mirabilis*, *Citrobacter* spp. and *S. typhi* (1:1). A Chi-square analysis of gender-wise species distribution indicated that there is no statistically significant difference between prevalence rates for the occurrence of gram negative pathogens between male and female patients.

The highest prevalence rates for the occurrence of *P. oryzae*, *M. morganii*, *A. baumannii*, *P. mirabilis*, *Citrobacter* spp., *S. typhi*, *E. coli* and *P. aeruginosa*, were within the age group 41-65 years, being 100%, 67%, 61%, 50%, 50%, 50%, 37.8% and 37.2%, respectively whereas the highest percentage of patients (48%) affected with a bacterial infection caused by *K. pneumoniae* and *E. cloacae* belonged to the age group 20-40 years. The lowest prevalence rates for the occurrence of gram negative bacterial pathogens was observed in the age group <1 year.

A clinical samples-wise distribution of Gram negative bacterial pathogens is also varied. The highest percentage (54.25%) of Gram negative bacterial pathogens was recovered from urine samples obtained from patients affected with urinary tract infections (UTIs). This was followed by pus samples (33.43%). The lowest distribution (0.29%) of gram negative bacterial pathogens was found in stool sample, followed by CSF, semen, throat swab, breast milk, sputum, ear swab and blood. The comparative antibiotic susceptibility profiles of clinical isolates of gram negative bacteria from Enterobacteriaceae against commonly used antibiotics in the bacterial infections are summarized in the Table III. The comparative antibiotic susceptibility profiles of clinical isolates of gram negative bacteria from non-Enterobacteriaceae against commonly used antibiotics in the bacterial infections are summarized in the Table IV.

TABLE III.- COMPARATIVE ANTIBIOTIC SUSCEPTIBILITY PROFILES OF CLINICAL ISOLATES OF GRAM NEGATIVE BACTERIA FROM NON-ENTEROBACTERIACEAE.

Antibiotics	B. capacia		P. oryzae		A. baumannii		P.aeruginosa	
	R%	S%	R%	S%	R%	S%	R%	S%
GEN	100	0	100	0	69	31	23	77
AMK	0	100	100	0	77	23	0	100
NET	0	0	0	0	0	0	100	0
MEM	0	100	100	0	92	8	16	84
IPM	0	0	100	0	92	8	14	86
CEC	0	0	0	0	0	0	0	0
CFP	0	100	0	0	100	0	100	0
CTX	0	0	0	0	100	0	100	0
CAZ	0	100	0	0	100	0	100	0
CFM	0	0	100	0	100	0	0	0
CRO	0	0	100	0	100	0	100	0
CTT	0	0	0	0	100	0	3	97
FEP	0	100	100	0	100	0	19	81
SCF	0	100	0	0	85	15	100	0
DAL	0	0	0	0	0	0	0	0
VAN	0	0	0	100	0	0	0	0
CHL	0	0	100	0	0	0	0	0
TGC	0	0	0	0	0	100	0	0
FOS	0	0	0	0	0	0	0	100
TEC	0	0	0	0	0	0	0	0
ERY	0	0	0	0	0	0	0	0
NIT	0	0	0	0	0	0	0	0
LZD	0	0	0	100	0	0	0	0
AMP	0	0	100	0	100	0	100	0
MET	0	0	0	0	0	0	0	0
PIP	0	0	0	0	100	0	0	0
AMC	0	0	100	0	100	0	100	0
TZP	0	100	0	0	100	0	28	72
PMB	0	0	0	0	100	0	3	97

The comparative antibiotic susceptibility profiles of clinical isolates of gram negative bacteria from Enterobacteriaceae and non-Enterobacteriaceae against commonly used antibiotics in the bacterial infections are summarized in the Table III. The overall MAR indices for each antibiotic against the pathogenic isolates of gram negative bacteria are summarized in the Table V. The comparative MAR indices for each antibiotic against the pathogenic isolates of gram negative bacteria from Enterobacteriaceae and non-Enterobacteriaceae are

summarized in the Figure 4. The Chi-square analysis of MAR indices for each antibiotic against the pathogenic isolates of gram negative bacteria did not indicate any significant difference among them ($P = 0.70$).

TABLE IV.- COMPARATIVE ANTIBIOTIC SUSCEPTIBILITY PROFILES OF CLINICAL ISOLATES OF GRAM NEGATIVE BACTERIA FROM ENTEROBACTERIACEAE AND NON- ENTEROBACTERIACEAE.

Antibiotics	Enterobacteriaceae		Non-Enterobacteriaceae	
	R%	S%	R%	S%
GEN	40.984	59.016	73.333	26.667
AMK	75.203	24.797	73.333	26.667
NET	32.061	67.939	0.000	100.000
MEM	14.523	85.477	86.667	13.333
IPM	12.766	87.234	92.857	7.143
CEC	62.500	37.500	0.000	100.000
CFP	97.076	2.924	92.308	7.692
CTX	100.000	0.000	100.000	0.000
CAZ	98.089	1.911	90.909	9.091
CFM	72.368	27.632	100.000	0.000
CRO	68.898	31.102	100.000	0.000
CTT	38.462	61.538	100.000	0.000
FEP	69.767	30.233	93.333	6.667
SCF	18.824	81.176	78.571	21.429
DAL	50.000	50.000	0.000	100.000
VAN	100.000	0.000	0.000	100.000
TEC	100.000	0.000	0.000	100.000
ERY	0.000	100.000	0.000	100.000
NIT	50.000	50.000	0.000	100.000
LZD	66.667	33.333	0.000	100.000
AMP	91.701	8.299	100.000	0.000
MET	33.333	66.667	0.000	100.000
PIP	44.444	55.556	100.000	0.000
AMC	77.236	22.764	100.000	0.000
TZP	34.783	65.217	92.857	7.143
PMB	27.907	72.093	100.000	0.000
CIP	74.897	25.103	85.714	14.286
ENX	99.398	0.602	91.667	8.333
OFX	100.000	0.000	100.000	0.000
LEV	88.732	11.268	100.000	0.000
NOR	74.483	25.517	0.000	100.000
MXF	83.333	16.667	100.000	0.000
SXT	68.776	31.224	83.333	16.667
MIN	66.667	33.333	0.000	100.000
DOX	72.277	27.723	42.857	57.143
CHL	33.333	66.667	100.000	0.000
TGC	0.000	100.000	0.000	100.000
FOS	10.000	90.000	0.000	100.000

TABLE V.- OVERALL MAR INDICES OF THE PATHOGENIC ISOLATES OF GRAM NEGATIVE BACTERIA.

Antibiotics	Overall Patterns
GEN	0.011
AMK	0.020
NET	0.008
MEM	0.005
IPM	0.005
CEC	0.016
CFP	0.025
CTX	0.026
CAZ	0.026
CFM	0.019
CRO	0.019
CTT	0.013
FEP	0.019
SCF	0.006
DAL	0.013
VAN	0.013
TEC	0.026
ERY	0.000
NIT	0.013
LZD	0.013
AMP	0.024
MET	0.009
PIP	0.014
AMC	0.021
TZP	0.010
PMB	0.011
CIP	0.020
ENX	0.026
OFX	0.026
LEV	0.024
NOR	0.020
MXF	0.023
SXT	0.018
MIN	0.018
DOX	0.018
CHL	0.013
TGC	0.000
FOS	0.003

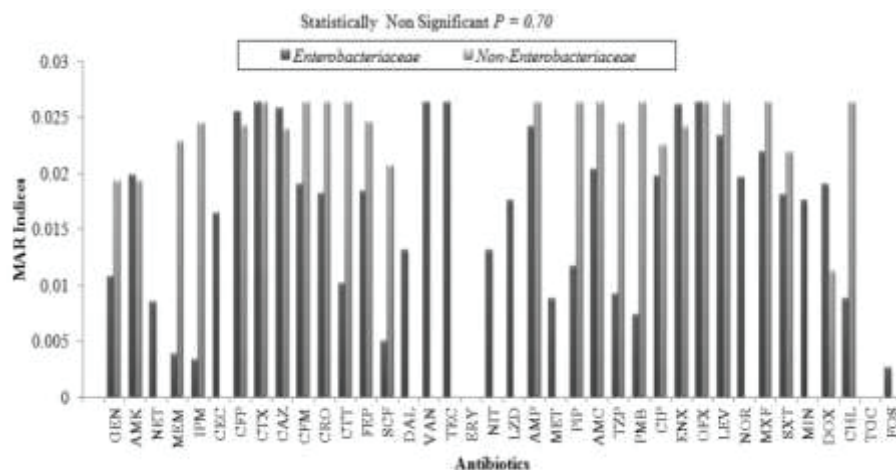


Fig. 4. Comparative MAR indices of pathogenic isolates of gram negative bacteria from Enterobacteriaceae and non-Enterobacteriaceae.

DISCUSSION

The global rise in antibiotic resistance patterns of gram negative bacterial pathogens has been well documented. This study has overviewed on the locally prevalent antibiotic susceptibility profiles of Gram negative pathogens isolated from various clinical samples and has correlated the data with patients' demographics. This will, therefore, provide a valuable data to compare and monitor the status of antibiotic resistance among gram negative bacterial pathogens to improve the empirical treatment in Rawalpindi and Islamabad.

We have found a predominance of bacterial infections in females and in patients aged 40-65 years. Our results agree with another previous research that showed that occurrence of urinary tract infections caused by Gram negative bacteria was higher amongst elder people; above forty eight years than youngsters (Prakash and Saxena, 2013).

A comparatively higher incidence of bacterial infections caused by Gram negative bacteria, especially members of Enterobacteriaceae is related to various factors that mediate their attachment to the uroepithelium as well as to their ability to colonize in the urogenital mucosa with adhesins, pili, fimbriae and P-1 blood group phenotype receptor (Das et al., 2006). *E. coli* was the most frequently isolated Gram negative bacteria in the positive clinical samples. These results are in line with most of reports from the globe (Foxman, 2003; Prakash and Saxena, 2013; Rudramurthy et al., 2015; Nerurkar et al., 2012); however, a

few reports have also stated *P. aeruginosa* (Ehinmidu et al., 2003) and *K. pneumoniae* (Aboderin et al., 2009) to be the most prevalent Gram negative bacterial species causing bacterial infection.

Gram negative bacteria showed (100 %) resistant to tigecycline, (66%) minocycline and (66%) to doxycycline. Gill et al. (2015) reported that about (56.4%) of the isolates were *E. coli*, (28.2%) were *K. pneumoniae*, (10.26%) were *Enterobacter* species, and (2.6%) were *Acinetobacter* species. Extended spectrum beta lactamases were found to be most sensitive to tigecycline, intermediate in susceptibility to minocycline while least sensitive to doxycycline and tetracycline.

In our study AMP showed overall 92.15 % resistant against Gram negative bacilli. The results of a previous study by Zaman et al. (2015) also revealed that Gram negative bacteria have high degrees of resistance to normally used antibiotics. Consideration would be made to the use of ampicillin which shows prodigious levels of antibiotic resistance against gram negative bacteria which was greater than 50 percent. The current study has also reported that 62 percent of Gram negative isolates were susceptible against piperacillin-Tazobactam. Likewise, previous reports has also showed that piperacillin-Tazobactam ensured outstanding medical effectiveness against Gram negative bacterial infections and proved a favorable beta lactamase inhibitor (Umadevi et al., 2011; Niki, 2001; Baby et al., 2008).

CONCLUSION

Present study provides a valuable data to compare and monitor the status of antibiotic resistance among Gram negative bacterial pathogens to improve the empirical treatment in Rawalpindi and Islamabad. Further research as the constant investigation of antibiotics vulnerability design of Gram negative bacteria is vital at different localities that can then afford support in management of operational preliminary treatments.

REFERENCES

- ABODERIN, O. A., ABDU, A. R., ODETOYIN, B. W. AND LAMIKANRA, A., 2009. Antimicrobial resistance in *Escherichia coli* strains from urinary tract infections. *J. Nat. med. Assoc.*, **101**: 1268-1273.
- AL'OS, J. I., 2005. Epidemiology and etiology of urinary tract infections in the community. Antimicrobial susceptibility of the main pathogens and clinical significance of resistance. *Enferm. Infecc. Microbiol. Clin.* **23**:3-8.

- BOUCHER, H. W., TALBOT, G. H., BRADLEY, J. S., EDWARDS, J. E., GILBERT, D., RICE, L. B., SCHELD, M., SPELLBERG, B. AND BARTLETT, J., 2009. Bad bugs, no drugs: no ESKAPE! An update from the infectious diseases Society of America. *Clin. Infect. Dis.*, **48**: pp.1-12.
- EHINMIDU, J. O., BOLAJI, R. O. AND ADEGBOYE, E. E. A., 2004. Isolation and antibiotic susceptibility profile of *Neisseria gonorrhoeae* isolated from urine samples in Zara, Northern Nigeria. *J. Phytomed. Therap.*, **9**.
- FOXMAN, B. AND BROWN, P., 2003. Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. *Infect. Dis. Clin. N. Am.*, **17**: 227-241.
- GILL, M. M., USMAN, J., HASSAN, A., KALEEM, F. AND SHAHEEN, N., 2015. In vitro susceptibility pattern of extended spectrum β -lactamase producing Gram negative bacilli against tetracyclines. *J. Ayub Med. Coll. Abbottabad*, **27**: 788-790.
- NERURKAR, A., SOLANKY, P. AND NAIK, S. S., 2012. Bacterial pathogens in urinary tract infection and antibiotic susceptibility pattern. *J. Pharm. Biomed. Sci.*, **21**: 1-3.
- NIKI, Y., 2001. Fundamental and clinical studies on beta-lactamase inhibitors. *Nippon. Rinsho*. **59**:771-776.
- PADMINI, S. B., RAJU, B. A. AND MANI, K. R., 2008. Detection of Enterobacteriaceae producing CTX-M extended spectrum β -lactamases from a tertiary care hospital in south India. *Indian J. med. Microbiol.*, **26**: 163.
- PELCZAR, M. J., CHAN, E. C. S. AND KRIEG, N. R. 1999. Host-parasite interaction; nonspecific host resistance. in: *Microbiology concepts and applications*.
- PELEG, A. Y. AND HOOPER, D. C. 2010. Hospital-acquired infections due to gram-negative bacteria. *N. Engl. J. Med.*, **362**: 1804-1813.
- PRAKASH, D. AND SAXENA, R. S., 2013. Distribution and antimicrobial Susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of meerut city, India. *ISRN Microbiol.*, 2013.
- RUDRAMURTHY, K. G., KUMARAN, R. AND GEETHA, R. K., 2015. Etiology and antimicrobial susceptibility pattern of bacterial agents from urinary tract infection in a tertiary care centre. *Int. J. Sci. Stud.* **2**: 125-127.
- TAMBEKAR, D. H., TAMBEKAR, S. D., JADHAV, A. S. AND BABHULKAR, B. V., 2016. Isolation and partial characterization of protease from *Bacillus halodurans* (aj302709) from alkaline lonar lake. *Int. J. Pharmaceut. Sci. Res.*, **7**: 4546.
- UMADEVI, S., KANDHAKUMARI, G., JOSEPH, N. M., KUMAR, S., EASOW, J. M., STEPHEN, S. AND SINGH, U. K., 2011. Prevalence and antimicrobial susceptibility pattern of ESBL producing gram negative bacilli. *J. Clin. Diagn. Res.*, **5**: 236-39.
- YEZLI, S., SHIBL, A. M., LIVERMORE, D. M. AND MEMISH, Z. A., 2014. Prevalence and antimicrobial resistance among Gram-negative pathogens in Saudi Arabia. *J. Chemother.*, **26**: 257-272.
- ZAMAN, R. M., ALY, M. M. AND HELMI, N. R., 2015. Antimicrobial susceptibility pattern of Gram-negative bacilli isolated from a teaching hospital in Jeddah, Saudi Arabia. *Afri. J. microbiol. Res.*, **9**: 2145-2158.

**TOLERANCE ABILITY OF POTATO CULTIVARS AGAINST
MYZUS PERSICAE (HOMOPTERA: APHIDIDAE)
UNDER GLASSHOUSE CONDITIONS**

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Abstract.- Tolerance is the resistive ability of plants to withstand herbivores attack to avoid growth arrest and production losses in plants. To test tolerance ability of eight different potato cultivars to *M. persicae* in a repeated experiment in 2015 and 2016, pairs of plants were made in completely randomized design. A single potato plant of each of the eight potato cultivars was infested with 30 aphids and pair plant of all cultivars were leave uninfested and enclosed in cages made by nylon mesh. The caged plants were removed after 15 days or were removed when they showed visual leaf symptoms (e.g. rolling and curling). The plants were cut at soil surface, pouched in aluminum foil and dry for 72 hours in an oven at 60 ± 4 °C then weighed. Comparisons of proportional plant dry weight change (DWT), tolerance index (TI) and SPAD index (SI) were used to determine tolerance component to *M. persicae* in eight potato cultivars. The cultivar Asterix showed highest tolerance by 49.29 g DWT followed by cultivar SH-5 (45.94 g DWT) to *M. persicae*, while the lowest tolerance was recorded both for cultivar Patrones (36.65 g DWT) and Desiree (36.94 g DWT). The maximum TI values were found for Sahiwal Red (3.88) and the minimum for cultivar Patrones (2.67). The highest SI was recorded for Asterix (55.10 %) and lowest for Patrones (35.75 %) in experiment in 2015. In 2016, the highest DWT were recorded for Asterix (48.37 g), the maximum TI value for Sahiwal Red (3.62) and SI for Desiree (63.97). The lowest DWT for Sahiwal Red (40.18 g), the minimum TI for SH-5 (2.97) and SI for Patrones (36.79). The DWT of most of the potato cultivars showed positive significant correlation with the TI and SI values. FD – 70, Sarco Mira and SH - 5 showed highly positive correlation ($p < 0.01$) of DWT with TI and SI with correlation coefficient values (0.948, 0.922 and 0.847), respectively. The data showed tolerance ability of the tested cultivars against *M. persicae*. Future research will be useful to compare these cultivars with earlier identified tolerant cultivars.

Key Words: Potato, *M. persicae*, Tolerance, SPAD, Resistance, Cultivars

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to family Solanaceae and originates from the South America. It occupies a prominent position among vegetable crops in Pakistan. Potato is grown globally in diverse agro-climatic conditions (Awan *et al.*, 2010; Hussain *et al.*, 2013) but it grows well in the temperate climates around the world. In Asia potato production is mainly coming

from the developing nations. The area under cultivation in Pakistan was 165.7 thousand hectare and in Khyber Pakhtunkhwa province was 9.11 thousand hectare while the average tuber production in Pakistan and KPK was 2883.8 and 105.6 thousand tons, respectively during 2014-2015 (Hussain *et al.*, 2013; NFS&R, 2015).

Various pests such as diseases, weeds and particularly the attack of aphids is considered to be one of the most serious issue of potato crop in Khyber Pakhtunkhwa as well as in rest of the provinces of Pakistan. Insect pests can cause 10 to 80% production losses to many crops globally, depending on insect pests population dynamics and crop management (Oerke, 2006; Bruce, 2010; Ferry and Gatehouse, 2010). Among insect pest the *M. persicae* is one of the most important pests belong to order Homoptera; *Aphididae*, the other pests of the Homoptera are stinkbugs (Pentatomidae), whiteflies (Aleyrodidae) and plant hoppers (Cicadellidae). Insect species of Homoptera can cause significant damage to the crops by different mode of activities such as by sucking sap of leaves which leads to chlorophyll loss. Insect species of this order are also capable to rapidly reproduce and increase their population size and thus to transmit diseases more efficiently.

Many insect pests of Homoptera are capable to develop resistance against insecticides (Painter, 1951; Panda and Khush, 1995). The development of insecticides resistance in *M. persicae* the early research work were published in 1955 (Anthon, 1955) against several major classes of insecticides including organophosphates, pyrethroids, carbamates, cyclodienes, and neonicotinoids (Bass *et al.*, 2014; Mottaghinia *et al.*, 2011). The present experiment was designed to test resistance of potato cultivars and to screen out the important component of tolerance. Tolerance is a distinctive category of resistance, having no effect on insect behaviour or biology, stability or withstand with insect injury due to which gained attention of researchers.

The present experiment was designed to test resistance of potato cultivars against a very commonly found insect *M. persicae*. To increase crop production and bring down infestation levels of *M. persicae* and minimize losses to potato crop, further research efforts are required. Such an effort could be achieved by identification of natural inherent traits of plants which are useful to resist insect or tolerate pest attack useful in reducing crop production losses. Tolerance is a useful trait of crops against herbivore attack (Pedigo and Rice, 2005). Tolerance is a distinctive category of resistance, having no effect on insect behaviour or

biology, stability or withstand with insect injury due to which gained attention of researchers. Different crops are reported to have resistance ability confer by different growth traits upon the crop plants against different insect pests attack (Herms and Mattson, 1992). Growth traits such as the rapid growth and development of plants (Mauromicale *et al.*, 2006; Busato *et al.*, 2010) and leaf chlorophyll contents can be useful in aiding resistance ability to the crop plants (Ahrens *et al.*, 1981; Khayatnezhad *et al.*, 2011). Growth of plants can be determined by measurement of shoot biomass production and leaf chlorophyll contents of plants can be measured by SPAD chlorophyll meter. Measurement of chlorophyll loss in potato infested and uninfested plants can be done by SPAD chlorophyll meter. This device can give an accurate measurement of chlorophyll content in plants (Inada, 1963; Vos and Bom, 1993). In earlier studies chlorophyll contents of potato was measured using SPAD (Gianquinto *et al.*, 2004; Olivier *et al.*, 2006; Li *et al.*, 2012). SPAD was also used by many other researchers for chlorophyll contents measurement other crops (Singh *et al.*, 2002).

The present study was undertaken under glasshouse conditions with specific objectives i) to test the resistance of eight different potato cultivars in terms of their proportional plant dry weight change and tolerance index against *M. persicae* and ii) find the relationship between *M. persicae* infestation levels and SPAD index of potato plants.

MATERIALS AND METHODS

Experiment setting and implementation

To test tolerance of eight different potato cultivars to *M. persicae*, two repeated experiments were conducted in 2015 and 2016 under glasshouse conditions in institute of biotechnology and genetic engineering, The University of Agriculture, Peshawar, Pakistan. The eight potato cultivars used were Desiree, Patrones, Rocco, Sarco Mira, Asterix, SH-5, FD-70 and Sahiwal Red. Plants of each of the eight cultivars were grown in pots (25 cm diameter) filled with potting mixture until their growth was reached to four leaves and then were individually enclosed in cages made of nylon mesh cloth. For each of the eight potato cultivars, 10 pairs of plants were created, a plant was infested with adult of *M. persicae* (30 per plant) and a pair plant was left uninfested (control) for comparison. The *M. persicae* were allowed for 15 days feeding or susceptible plants showed 95% symptoms of chlorosis. Finally, all plants were cut at soil

surface, dried for 72 hours in an oven ($60\pm4^{\circ}\text{C}$), weighed and determine above ground plant biomass.

Experimental procedures and design

The repeated experiment in 2015 and 2016 was including eight potato cultivars (treatments) and each treatment being replicated 10 times. The treatments were arranged in a completely randomized design. The expression of tolerance of potato plants to *M. persicae* was calculated from dry shoot biomass as proportional plant dry weight change; $\text{DWT} = [(\text{WC} - \text{WT}) / \text{WC}] \times 100$], where WC is weight of control plants and WT is dry weight of infested plants as has been calculated by (Reese *et al.*, 1994). The TI of potato cultivars was determined by counting of *M. persicae* present on each of the test plants of all eight cultivars to identify the most tolerant cultivars ($\text{TI} = \text{DWT} / \text{No of aphids produced on infested plants}$) (Reese *et al.*, 1994). The cultivars with high mean TI and DWT values were considered to be tolerant while the cultivars with low values were considered to be intolerant to *M. persicae*. The means of each of the replicated treatment were analyzed by applying TUKEY's test in statistical software STATISTIX 8.1.

To record the chlorophyll content, a SPAD- 502 (Minolta Co., Tokyo, Japan) was used where leaves of all test plants infested with *M. persicae* and those not infested were all read by the SPAD meter and recorded the values. The aphids were then removed, and the difference between the leaves of infested and uninfested (control) plants SPAD index values were recorded. The values were then put in formula: $\text{SPAD index} = \text{C} - \text{T} / \text{C}$ where C is SPAD unit value of control (noninfested) leaf tissue, and T is SPAD unit value of infested leaf tissue. The SPAD index can be shown either as a decimal points or as a percentage, by multiplying the decimal by 100. Five representative SPAD unit measurements were taken from each leaf and their means were calculated. Potato cultivars with a significantly lower chlorophyll content than the respective compared cultivar were considered tolerant, based on the methods of (Girma *et al.*, 1998).

RESULTS

The results of proportional plant dry weight change (DWT) and tolerance index (TI) values when leaves of potato cultivars were infested with *M. persicae* up to 15 days was statistically non-significant while SPAD index (SI) values of different potato cultivars showed significant difference ($P > 0.05$; Table I). All the

potato cultivars showed mixed response of resistance to the aphid *M. persicae* in terms of DWT. However, some cultivars such as Asterix gave highest (49.29 g DWT) and SH-5 (45.94 g DWT) while others such as Patrones (36.65 g DWT) and Desiree (36.94) gave lowest values (Table I).

Similarly, TI values were shown to be non-significant among the cultivars tested against the *M. persicae* (Table I). However, Sahiwal Red (3.88) and Asterix (3.87) showed higher values than those of the other remaining cultivars. The minimum TI values were found for Patrones (2.67) and Sarco Mira (3.9) against *M. persicae* infestation.

Interestingly, the SI values of the tested potato cultivars showed varied response to the *M. persicae* infestation (Table I). Two cultivars Asterix (55.10) and SH-5 (52.31) showed the highest SI values while Patrones (35.75) and Sahiwal Red (45.57) showed the lowest SI values.

TABLE I.- MEAN \pm SE PROPORTIONAL PLANT DRY WEIGHT CHANGE (DWT), TOLERANCE INDEX (TI) AND SPAD INDEX (SI) VALUES OF DIFFERENT POTATO CULTIVARS AGAINST *M. PERSICAE* IN 2015.

Potato cultivars	DWT	TI	SI
Desiree	36.94 \pm 5.81a	3.27 \pm 0.20a	51.54 \pm 16.5 ab
Patrones	36.65 \pm 4.79 a	2.67 \pm 0.60 a	35.75 \pm 9.0 b
Rocco	43.59 \pm 7.55a	3.09 \pm 0.85a	50.43 \pm 5.3 ab
Sarco Mira	39.13 \pm 5.22 a	3.04 \pm 0.68a	48.63 \pm 7.8 ab
Asterix	49.29 \pm 12.17 a	3.87 \pm 0.85a	55.10 \pm 6.0 a
SH – 5	45.94 \pm 7.54a	3.10 \pm 0.64a	52.31 \pm 19.3 ab
FD – 70	38.01 \pm 11.37a	3.13 \pm 1.23a	50.24 \pm 10.5 ab
Sahiwal Red	42.48 \pm 17.07a	3.88 \pm 1.51a	45.57 \pm 20.2 ab

Means followed by different small letter in columns are significantly different ($\alpha < 0.05$).

The results of DWT, TI and SI values are presented in Table II, when plants of potato cultivars were infested with *M. persicae* up to 15 days, DWT data showed non-significant difference among the tested cultivars to *M. persicae*. However, some cultivars Asterix (48.37 g) and Desiree (43.24 g) gave the highest values while Sahiwal Red (40.18 g) and SH-5 (40.91 g) gave the lowest values. The Sahiwal Red and Desiree gave the maximum TI values by 3.62 and 3.34, respectively while SH-5 and FD – 70 gave the minimum values by 2.97 and 3.07 against the *M. persicae*, respectively (Table II).

The SI for different potato cultivars was noted to show variable response to *M. persicae*. However, Desiree (63.97) and Rocco (53.96) both having the maximum SI values while Patrones (36.79) and FD-70 (37.23) both having the minimum SI values (Table II).

TABLE II.- MEAN \pm SE PROPORTIONAL PLANT DRY WEIGHT CHANGE (DWT), TOLERANCE INDEX (TI) AND SPAD INDEX (SI) VALUES OF DIFFERENT POTATO CULTIVARS AGAINST *M. PERSICAE* IN 2016.

Potato cultivars	DWT	TI	SI
Desiree	43.24 \pm 9.50 a	3.34 \pm 0.41a	63.975 \pm 4.3 a
Patrones	43.19 \pm 4.19a	3.27 \pm 0.87a	36.799 \pm 1.7 c
Rocco	41.64 \pm 1.98 a	3.20 \pm 0.49a	53.966 \pm 1.5 ab
Sarco Mira	42.95 \pm 12.69 a	3.32 \pm 0.94a	48.712 \pm 3.8 abc
Asterix	48.37 \pm 11.15a	3.08 \pm 1.08a	52.221 \pm 9.0 abc
SH – 5	40.91 \pm 7.68 a	2.97 \pm 0.52a	53.772 \pm 12.0 ab
FD – 70	43.06 \pm 9.80 a	3.07 \pm 0.75a	37.238 \pm 23.3 c
Sahiwal Red	40.18 \pm 20.06 a	3.62 \pm 1.43a	43.003 \pm 16.3 bc

Means followed by different small letter in columns are significantly different ($\alpha < 0.05$).

Relationship of plant dry weight change with tolerance index and chlorophyll content

Results showed significant positive correlation of DWT ($p < 0.05$) with TI and SI in most cultivars in experiments undertaken in 2015 and 2016 (Table III). In some cultivars including FD – 70, Sarco Mira and SH - 5 the DWT showed highly positive correlation ($p < 0.01$) with TI and SI and showed correlation coefficient values by 0.948, 0.922 and 0.847, respectively in the experiment done in 2016 (Table III). The positive correlation of DWT with TI and SI values was more pronounced in the experiment undertaken in 2016 than the experiment undertaken in 2015 (Table III). Sarco Mira, FD – 70 and Sahiwal red showed positive correlation of DWT with TI and SI in 2015 and Patrones, Sarco Mira, Asterix, SH – 5 and FD – 70 all showed positive correlation in 2016. On the other hand Desiree, Rocco and SH – 5 showed negative correlation of DWT with TI and SI in 2015 and Sahiwal red in 2016 (Table III).

TABLE III.- CORRELATION OF PROPORTIONAL PLANT DRY WEIGHT CHANGE WITH TOLERANCE INDEX AND SPAD INDEX OF EIGHT DIFFERENT POTATO CULTIVARS.

Cultivars	Year – 2015		Year - 2016	
	Tolerance index	SPAD index	Tolerance index	SPAD index
Desiree	-0.406 (0.245)	-0.349 (0.323)	-0.149 (0.680)	0.121 (0.740)
Patrones	0.129 (0.722)	-0.287 (0.421)	0.200 (0.580)	0.165 (0.648)
Rocco	-0.608 (0.062)	-0.462 (0.179)	0.699* (0.025)	-0.237 (0.510)
Sarco Mira	0.710* (0.022)	0.243 (0.499)	0.922** (0.000)	0.236 (0.512)
Asterix	0.215 (0.550)	-0.433 (0.212)	0.295 (0.408)	0.165 (0.648)
SH – 5	-0.218 (0.544)	-0.638* (0.047)	0.847** (0.002)	0.339 (0.338)
FD – 70	0.941* (0.000)	0.000 (0.999)	0.948** (0.000)	0.278 (0.437)
Sahiwal Red	0.123 (0.735)	0.371 (0.291)	-0.012 (0.974)	-0.630 (0.051)

The values in parenthesis are the p-values of t-ratio for correlation coefficient; * and ** show significant correlation at 5% and 1% level of significance, respectively.

DISCUSSION

As host plant potato is one of the suitable plant species for different insect pests, however varietal response as host plant could be different depending on their growth and morphological characteristics (Frei *et al.*, 2003; Mottaghinia *et al.*, 2010). Different cultivars of potato may also possessing different quantities of bio-chemicals; all such plant features are useful in resistance capability promotion against insect pests attack (Ave and Tingey, 1986; Mottaghinia *et al.*, 2010). Assessing resistance capabilities of different cultivars of potato in terms of growth parameters against insect pests could give valuable information to minimize the losses by insect pests.

Myzus persicae being an important insect pest of potato crop can cause significant losses to the crop by sucking leaf phloem sap which negatively affects the photosynthetic process (Busato *et al.* 2010). Although resistance ability among the eight tested potato cultivars in terms of DWT and TI against *M. persicae* was shown to be non-significant but with mixed response (Tables I and II). This response of the tested potato cultivars to *M. persicae* may be due to their equal resistance ability against *M. persicae*. However, if the aphid infestation was to be prolonged beyond 15 days, there may be a significant diverse effect of *M. persicae* on the potato plants as has been recorded by Minotti *et al.* (1994) and

Gil *et al.* (2002). Visual symptoms of leaves chlorosis of tested cultivars of potato noted during the study and at four leaf growth stages can provide a general effect of *M. persicae* infestation. However, this initial effect of *M. persicae* may have been compensated and recovered by the potato plants in later growth stages as has been previously reported by Nagaraj *et al.* (2005). Tolerance can measure one of the focal categories of resistance aphid (Velusamy and Heinrichs, 1986; Dixon, 1987). A number of sorghum varieties are reported to possess tolerance against the attack of aphids (Teetes, 1980). Such bio-chemical characteristics of plants are thought to play a useful role as tolerance and can act as biological control of insect pests (Reed *et al.*, 1991; van Lentern *et al.*, 1991; Riggins *et al.*, 1992).

Interestingly SI values recorded for the eight different cultivars of potato have shown to be significantly different (Tables I and II). Three cultivars Desiree, Rocco and SH – 5 showed highest SI values compared to the remaining five cultivars. This significant difference of SI values indicates that these three cultivars are greatly tolerant to the attack of *M. persicae*. This tolerance capability may be due to the phyto-chemicals present in these three cultivars as has been reported by (Stamp, 2003) or due to the presence of greater trichomes number on the leaves of plants. However, Flinn *et al.* (2001) reported such an ability of tolerance to be useful in increasing population of beneficial insects present on host plants.

The positive correlation of DWT with TI and SI of the test plants of eight different potato cultivars show that the TI and SI increase or decrease could lead to the proportional dry weight change in plants infested by the *M. persicae*. Similar results have been reported in earlier studies (Samdur *et al.*, 2000; Costa *et al.*, 2001; Goławska *et al.*, 2010). Those potato cultivars which showed positive correlation among its different growth parameter could be useful in aiding into their tolerance ability against the attack of *M. persicae*. This ability of such potato cultivars may have an important implication in future management programmes for *M. persicae* infestations in potato growing areas in Khyber Pakhtunkhwa and in rest of the country.

It can be concluded from the study that identification of tolerant potato cultivars against *M. persicae* could be an effective and environment friendly management tool in aiding to the existing control approaches. Those potato cultivars which are tolerant to insect pests attack are may be unique as such cultivars helps in increasing and supporting natural enemies parasites and

predators. Tolerant potato cultivars can also be integrated with other biological control approaches to strengthen integrated pest management. As the present study was undertaken under glasshouse conditions, future studies will be required to test potato cultivars under natural field conditions for tolerance capability against *M. persicae*.

REFERENCES

- AHRENS, W.H., ARNTZEN, C.J. AND STOLLER, E.W., 1981. Chlorophyll fluorescence assay for the determination of triazine resistance. *Weed Sci.*, **29**: 316-322.
- ANTHON, E.W., 1955. Evidence for green peach aphid resistance to organophosphorous insecticides. *J. econ. Ent.*, **48**: 56-57.
- AVE, D.A. AND TINGEY, W.M., 1986. Phenolic constituents of glandular trichomes on *Solanum berthaultii* and *Solanum polydenium*. *Am. J. Potato Res.*, **63**: 473-480.
- AWAN, A. R., HAQ, I. BABAR M. E. AND NASIR, I. A., 2010. Molecular detection of potato leaf roll polerovirus through reverse transcription polymerase chain reaction in dormant potato tubers. *Pak. J. Bot.*, **42**: 3299-3306.
- BASS, C., PUINEAN, A. M., ZIMMER, C. T., DENHOLM, I., FIELD, L. M., FOSTER, S. P., AND WILLIAMSON, M. S., 2014. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochem. Mol. Biol.*, **51**: 41-51.
- BRUCE, T.J.A., 2010. Tackling the threat to food security caused by crop pests in the new millennium. *Fd. Sec.*, **2**: 133-141. doi:10.1007/s12571-010-0061-8
- BUSATO, C., FONTES, P. C. R., BRAUN, H. AND CECON, P. R., 2010. Seasonal variation and threshold values for chlorophyll meter readings on leaves of potato cultivars. *J. Pl. Nutri.*, **33**: 2148-2156.
- COSTA, C., DWYER, L.M., DUTILLEUL, P., STEWART, D.W., MA, L.B. AND SMITH, D.I., 2001. Inter-relationships of applied nitrogen, SPAD, and yield of leafy and non-leafy maize genotypes. *J. Pl. Nutri.*, **24**: 1173-1194.
- DIXON, A.F.G., 1987. Cereal aphids as an applied problem. *Agric. Zool. Rev.*, **2**: 1-57. doi: 10.1111/1744-7917. 12011.
- FERRY, N. AND GATEHOUSE, A.M.R., 2010. Transgenic crop plants for resistance to biotic stress, In: *Transgenic crop plants: Utilization and biosafety*, Vol. 2 (eds C. Kole, C.H. Michler, A.G. Abbott and T.C. Hall) Springer-Verlag, Berlin: pp. 1-66.
- FLINN, M., SMITH, C. M., REESE, J. C. AND GILL, B., 2001. Categories of resistance to greenbug (Homoptera: Aphididae) biotype I in *Aegilops tauschii* germplasm. *J. econ. Ent.*, **94**: 558-563.
- FREI, A., GU, H., BUENO, J.M., CARDONA, C. AND DORN, S., 2003. Antixenosis and antibiosis of common beans to *Thrips palmi* Karny (Thysanoptera: Thripidae). *J. econ. Ent.*, **93**: 1577-1584.
- GIANQUINTO, G., GOFFART, J.P., OLIVIER, M., GUARDA, G., COLAUZZI, M., DALLA COSTA, L., DELLE VEDOVE, G., VOS, J. AND MACKERRON, D.K.L., 2004. The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. *Potato Res.*, **47**: 35-80.

- GIL, P. T., FONTES, P. C. R. CECON, P. R. AND FERREIRA, F. A., 2002. SPAD index for nitrogen status diagnosis and potato yield prognosis. *Horticul. Brasil*, **20**: 611–615.
- GOŁAWSKA, S., KRZYŻANOWSKI, R. AND ŁUKASIK, I., 2010. Relationship between aphid infestation and chlorophyll content in Fabaceae species. *Acta Biol. Craco. Ser. Bot.*, **52**: 76–80.
- HERMS, D.A. AND MATTSON, W.J., 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.*, **67**: 283–335.
- HUSSAIN, Z., MUNSIF, F., MARWAT, K. B., ALI, K., AFRIDI, R. A. AND BIBI, S., 2013. Studies on efficacy of different herbicides against weeds in potato crop in Peshawar. *Pak. J. Bot.*, **45**: 487–491.
- INADA, K., 1963. Studies on a method for determining the deepness of green color and chlorophyll content of intact crop leaves and its practical applications. I. Principles of estimating the deepness of green color and chlorophyll content of whole leaves. *Proc. Sci. Soc., (Japan)*, **32**: 157–162.
- KHAYATNEZHAD, M., GHOLAMIN, R., JAMAATI-E-SOMARIN, S.H. AND ZABIHIE MAHMOODABAD, R., 2011. The leaf chlorophyll content and stress resistance relationship considering in corn cultivars (*Zea mays*). *Adv. environ. Biol.*, **5**: 118–122.
- LI, L., QIN, Y., LIU, Y., HU, Y. AND FAN, M., 2012. Leaf positions of potato suitable for determination of nitrogen content with a SPAD meter. *Pl. Produ. Sci.*, **15**: 317–322.
- MAUROMICALE, G., IERNA, A. AND MARCHESE, M., 2006. Chlorophyll fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age. *Photosynthetica*, **44**: 76–82.
- MINOTTI, P. L., HALSETH, D. E. AND SIECZKA, J. B., 1994. Field chlorophyll measurements to assess the nitrogen status of potato varieties. *Hortscience*, **29**: 1497–1500.
- MOTTAGHINIA, L., RAZMJOU, J., NOURI-GANBALANI, G. AND RAFIEE-DASTJERDI, H., 2011. Antibiosis and antixenosis of six commonly produced potato cultivars to the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae). *Neotrop. Ent.*, **40**: 380–386.
- MOTTAGHINIA, L., RAZMJOU, J., NOURI-GANBALANI, G. AND RAFIEE-DASTJERDI, H., 2011. Antibiosis and antixenosis of six commonly produced potato cultivars to the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae). *Neotrop. Ent.*, **40**: 380–386.
- NAGARAJ, N., REESE, J. C., TUINSTRA, M. R., SMITH, C. M., ST. AMAND, P., KIRKHAM, M. B. AND WILDE, G., 2005. Molecular mapping of sorghum genes expressing tolerance to damage by greenbug (Homoptera: Aphididae). *J. econ. Ent.*, **98**: 595–602.
- NATIONAL FOOD SECURITY AND RESEARCH OF PAKISTAN, 2015.
- OERKE, E.C., 2006. Crop losses to pests. *J. Agric. Sci.*, **144**: 31–43.doi: 10.1017/s0021859605005708
- OLIVIER, M., GOFFART, J. P. AND LEDENT, J. F., 2006. Threshold value for chlorophyll meter as decision tool for nitrogen management of potato. *Agron. J.*, **98**: 496–506.
- PAINTER, R. H., 1951. *Insect resistance in crop plants*. The University Press of Kansas. Lawrence, KS.
- PANDA, N. AND KHUSH, G.S., 1995. *Host plant resistance to insects*. Wallingford, CABI. CT.
- PEDIGO, L.P. AND RICE, M.E., 2005. *Entomology and pest management*. Upper Saddle River. NJ: Pearson Education, Inc.

- REED, D. K., WEBSTER, J. A. JONES, B. C. AND BURD, J. D., 1991. Tritrophic relationships of Russian wheat aphid (Homoptera Aphididae), a hymenopterous parasitoid (*Diaeretiella rapae* McIntosh), and resistant and susceptible small grains. *Biol. Contr.*, **1**: 35-41.
- RIGGIN, T. M., WISEMAN, B. R. ISENHOUR, D. J. AND ESPELIE, K. E., 1992. Incidence of fall armyworm (Lepidoptera: Noctuidae) parasitoids on resistant and susceptible corn genotypes. *Environ. Ent.*, **21**: 888-895.
- SAMDUR, M.Y., SINGH A.L., MATHUR, R.K., MANIVEL, P., CHIKANI, B.M., GOR, H.K. AND KHAN, M.A., 2000. Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron-deficiency chlorosis. *Curr. Sci. Bangalore*, **79**: 211-214.
- SINGH, B., SINGH, Y., LADHA, J.K., BRONSON, K.F., BALASUBRAMANIAN, V., SINGH, J. AND KHIND, C.S., 2002. Chlorophyll meter- and leaf color chart-based nitrogen management for rice and wheat in northwestern India. *Agron. J.*, **94**: 821-829.
- STAMP, N., 2003. Out of the quagmire of plant defense hypotheses. *Q. Rev. Biol.*, **78**: 23-55. PMID: 12661508.
- TEETES, G. L., 1980. Breeding sorghums resistant to insects. pp. 451-485. In: *Breeding plants resistant to insect* (eds. F. G. Maxwell and P. R. Jennings), Wiley, New York.
- VAN LENTERN, J. C., 1991. Biological control in a tritrophic system approach. In: (eds. D. C. Peters and J. A. Webster). *Aphid-plant interactions: populations to molecule*. Oklahoma State University Press, Stillwater, OK.
- VELUSAMY, R. AND HEINRICHS, E. A., 1986. Tolerance in crop plants to insect pests. *Insect Sci. Applic.*, **7**: 689-696.
- VOS, J. AND BOM, M., 1993. Hand-held chlorophyll meter: a promising tool to assess the nitrogen status of potato foliage. *Potato Res.*, **36**: 301-308.

BIO-EFFICACY OF ETHYL ACETATE EXTRACTS FROM PLANT MATERIALS ON MAIZE WEEVIL (*SITOPHILUS ZEAMAI*S) (MOTSCHULSKY), (COLEOPTERA: CURCOLIONIDAE)

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Abstract.- Insect pests cause extensive quantitative and qualitative losses to stored grains and their value added products. Among the stored grain pests, *Sitophilus zeamais* (L.) is considered as one of the most destructive pests of stored cereals around the globe. Control of maize weevil is mainly achieved by the applications of fumigants. However, the excessive use of fumigants has led to the development of resistant populations, enhancing the need to develop alternative control measures. Plant derivatives are emerging as alternatives to traditional chemical insecticides as they carry rich sources of bioactive molecules. In the present study the ethyl acetate extracts of six different plants viz. Neem seed (*Azadirachta indica*), Bitter cress succulent fruit (*Caralluma turberculata*), Garlic rhizomes (*Allium sativum*), Turmeric rhizome (*Curcuma longa*), Tumba fruit (*Citrullus colocynthis*) and Ak leaves (*Calotropis procera*) at six concentrations (0.5, 1, 1.5, 2, 2.5 and 3%) were evaluated against maize weevil for their effects on days to F₁ emergence, inhibition of F₁ adult emergence, percent infestation and grain weight loss, adult longevity and sex ratio. The newly emerged ten pairs of adult weevils were exposed to the plant extracts by grain treatment test in 200ml transparent plastic jars at constant conditions of 27±2°C, 65± 5% R.H. The results revealed that all the plant extracts carry biological effects against maize weevil compared to control. The significantly longest developmental duration, minimum number of F₁ adult emergence, percent infestation and weight losses were observed in grains pre-treated with the highest concentration of *A. indica* extracts followed by *C. longa* extracts while the shortest developmental duration, highest incidence and maximum grain damage was observed in untreated maize grains. Moreover, all the tested extracts significantly reduced the adult longevity of maize weevil compared to control. Moreover, the shortest adult longevity of maize weevil was recorded in maize grains treated with 3% concentration of *A. indica* extracts whereas the longest adult longevity was recorded on untreated maize grains. The overall results of this study suggest that *A. indica* and *C. longa* extracts has potential insecticidal effects which might be used in pest control.

Keywords: Stored Maize Grain, maize weevil, Botanicals, Ethyl acetate extracts

INTRODUCTION

Maize (*Zea mays* L.) is considered as queen of cereal crops due to its versatility; which is eaten from flower to flour (Boutard, 2012). Being the 3rd

most important cereal crop globally following wheat and rice (Adarkwah *et al.*, 2012). It is the chief staple food and key source of calories in most developing countries (Hsiao *et al.*, 2009), a source of feed, biofuel and source of raw material for many industries. As per observations recorded by Food and Agriculture Organization (FAO), more than 800 million metric tons of maize was produced during 2010-2011 (Stricevic *et al.*, 2011); which is predicted to be doubled by 2025 and furthest crop in terms of production by 2050 (Rosegrant *et al.*, 2008).

Despite increased production; post-harvest grain damage due to biotic factors such as insects and molds is one of the major problems and a huge challenge throughout the world resulting in increased hunger and human labor (Tefera *et al.*, 2013). These damages can occur before and after harvesting during storage which lead to losses of food grain, qualitative and financial losses (López *et al.*, 2008). As per estimates provided by (Adebayo Ojo and Amos Omoloye, 2012) 14 to 50% of maize is lost due to insect infestation in developing countries every season, while 1 to 2% in developed countries.

The prime cause of these grain losses is maize weevil, *S. zeamais* (Motschulsky during storage period (Ukeh *et al.*, 2012). The maize weevil is among the most destructive pests in stored grains; regarded as internal feeder (Rees, 2004; Suleiman *et al.*, 2015). Adult female of maize weevil causes damage by making hole and laying eggs inside the kernel, later on larvae and pupae grow by eating the ingredients of the kernel, leading to damaged kernel having reduced grain weight (Adebayo Ojo and Amos Omoloye, 2012). The weevils, apart from causing weight losses, cause severe reductions in nutritive and economic standards, germination potential, are a source of contamination by chemical excretions (silk) and insect remains (Ukeh *et al.*, 2012). The infestation also raises temperature and moisture content of stored grains, which facilitates the development of various species of molds, such as *Aspergillus flavus* (Chu *et al.*, 2013).

Control of obnoxious insects remained consistently a challenging task since the beginning of modern agriculture. The use of chemical pesticides, mainly chlorinated hydrocarbons, insect pest's population remained under control to a certain level and it was presumed that the pest problem was resolved. Due to increasing problems of pests' resistance to these chemicals; increased cost of application, toxic residues and environmental pollution, fears for toxicity during application and residual effects on the preserved food (Adedire *et al.*, 2011;

Ashamo et al., 2013; Lekei et al., 2014; Ogungbite and Oyeniyi, 2014; Oni and Ileke, 2008; Opit et al., 2012; Stadlinger et al., 2011) it became unavoidable to find alternate sustainable and economical control measures which are cost effective and safe for all non-target organisms and environment.

The integrated pest management (IPM) strategies are promoting the use of alternative measures which are environmental friendly. The botanical pesticides; as promising alternatives to synthetic pesticides are safe and target specific, eco-friendly and cause no residual effects to non- target organisms (Dubey *et al.*, 2010; Olayinka-Olagunju, 2014; Zibae, 2011).

The studies were conducted to investigate the pesticidal potential of crude ethyl acetate extracts of six different plants viz. neem seed (*Azadirachta indica*), bitter cress succulent fruit (*Caralluma tuberculata*), garlic rhizomes (*Allium sativum*), turmeric rhizome (*Curcuma longa*), tumha fruit (*Citrullus colocynthis*) and Ak leaves (*Calotropis procera*) at six different concentrations, against maize weevil on stored maize. The findings from this study will help to indicate if ethyl acetate extracts of tested plants can be used as alternative to synthetic insecticides by subsistence farmers against maize weevil during storage conditions.

MATERIALS AND METHODS

Collection and preparation of plant materials

All the plant materials were collected from the local market of Dera Ismail Khan Pakistan. These plant materials were brought to laboratory of Entomology Department, Faculty of Agriculture Gomal University, Dera Ismail Khan. The plant materials were washed with tap water, cleaned and dried up for 7 days at room temperature under shade and powdered in electric grinder.

Extraction of collected plant powders were done in ethyl acetate by means of prescribed method (Okoye and Osadebe, 2009). The powdered materials of 300g for each plant materials were passed via mesh sieve (0.2mm) and dissolved in ethyl acetate solvent with ratio of (1:1). The solution was vigorously stirred and shaken on hourly basis extending from morning to evening. Maceration process continued for seven days for each plant material. After a week period all the extracts were filtered via muslin cloth and then passed via a Whatmen No. 1 filter paper. Using rotary evaporator filtrate were collected and concentrated to almost dryness. The crude extracts used for experiment were stored in

refrigerator. Using digital balance (KERN, 572; max cap 421g; readability 0.0001g), the extracts were accurately weight according to six concentrations i.e. 0.5, 1, 1.5, 2, 2.5 and 3%, respectively. The extracts were diluted in 3ml of respective solvent and mixed in sterilized maize seeds and were shaken well to facilitate coating of the extracts on maize grains. Before introducing the adult maize weevils in the plastic jars, all the samples were kept for 5h to allow the solvent to completely evaporate.

Experimental protocol

The experiment was conducted to find out bio-active effect of ethyl acetate plant extracts against maize weevil. The experiment was designed CRD with 5 replications for each treatment. Each concentration of ethyl acetate plant extract was mixed thoroughly with 20grams of sterilized maize seeds. The transparent plastic jar except the control were vigorously shaken for complete mixing of plant extracts prior to release of weevils. An hour period was maintained between the release of adult weevils and treatment of grains. Ten pairs of newly emerged weevils were selected on the basis of their morphological description and released in each jar. Before releasing in the test arena weevils were kept starved for one hour. Data was recorded on different parameters which are as follows:

Days to F_1 emergence, total number of F_1 adult emerged, percent infestation, percent weight loss, Sex ratio and total longevity of weevil (male and female).

Statistical analysis

All the recorded data were subjected to analysis of variance (ANOVA) and means of all experimental plant materials as treatments were divided by applying LSD test (least Significant Difference) at 0.05 significant level. One way analysis of variance (ANOVA) was used to find out the effect of treatments and concentrations against the experimental insect (maize weevil) during stipulated time period. All the data were analyzed by using computer software (STATISTX version 8.1)

RESULTS

Days to F_1 emergence

The studies carried out on the evaluation of plant powders along with the organic solvent ethyl acetate showed significant effect on the developmental

duration of maize weevil as compared to control. The organic solvent extracts delayed the maturity of the insect in *A. indica* and *C. longa* treated grains as compared to other treatments and control. The efficacy of plant extracts increased with the increase in the concentrations of plant extracts. The maximum efficacy of plant products was noted at highest concentrations of 3%, whereas; the minimum efficacy was noted at the lowest concentration of 0.5%. The maximum developmental duration (days) was recorded in *A. indica* extracts at 3% whereas; minimum developmental duration was recorded in untreated maize grains. Among the treatments, the least effective treatments were *C. procera* and *C. colocynthis*, having 27.80 and 32.20 days developmental duration although they were significant from control (Table I).

TABLE I.- EFFECT OF DIFFERENT CONCENTRATIONS OF ETHYL ACETATE EXTRACTS OF PLANT POWDERS ON THE DAYS (\pm SE) TO F_1 EMERGENCE OF *S. ZEAMAI*S REARED ON MAIZE GRAINS.

Treatments	Concentrations (%)					
	0.5	1	1.5	2	2.5	3
<i>Azadirachta indica</i>	35.60 \pm 1.14 a	36.00 \pm 0.70 a	37.40 \pm 1.14 a	38.00 \pm 1.22 a	39.00 \pm 0.89 a	38.20 \pm 0.83 a
<i>Caralluma tuberculata</i>	31.80 \pm 1.92 b	31.00 \pm 1.58 b	31.80 \pm 2.16 c	32.00 \pm 1.58 c	33.20 \pm 0.97 c	33.80 \pm 1.48 c
<i>Allium sativum</i>	34.00 \pm 0.70 a	34.60 \pm 0.89 a	35.20 \pm 1.64 b	35.80 \pm 1.78 b	36.20 \pm 1.16 b	36.80 \pm 1.78 b
<i>Curcuma longa</i>	34.60 \pm 1.14 a	34.80 \pm 1.30 a	36.40 \pm 0.54 ab	37.40 \pm \pm 1.14ab	38.20 \pm 1.46 a	38.40 \pm 1.34 a
<i>Citrullus colocynthis</i>	32.20 \pm 2.16 b	32.20 \pm 0.83 b	33.00 \pm 0.70 c	33.20 \pm 1.30 c	34.00 \pm 0.63 c	34.40 \pm 1.14 c
<i>Calotropis procera</i>	27.80 \pm 0.83 c	27.60 \pm 1.34 c	28.20 \pm 1.92 d	30.00 \pm 0.70 d	31.00 \pm 0.63 d	31.20 \pm 0.83 d
Control	24.80 \pm 0.44 d	24.80 \pm 0.44 d	24.80 \pm 0.44 e	24.80 \pm 0.44 e	24.80 \pm 0.44 e	24.80 \pm 0.44 e
LSD Value	1.72	1.40	1.79	1.61	1.36	1.55

Each value is a mean \pm standard error of five replications. Means followed by the same letters along the column are not significantly different at ($P>0.05$) using LSD Test.

Total number of F_1 adults emerged

The crude extracts of plant materials prepared in ethyl acetate caused significant reduction in progeny production of maize weevil relative to control, which was dose dependent. It was observed that, there was an inverse

relationship between the adult emergence and level of concentration of the plant materials (Table II). Among the tested plant extracts, *C. colocynthis* applied at 0.5% resulted in the highest (64.80) adult emergence while the least (21.20) was obtained from maize grains treated with 3% concentration of *A. indica* extracts followed by *C. longa* extracts at highest concentration. The effect of all the plant extracts at all the evaluated concentrations was significant compared to control. The highest number of adult emergence was recorded in untreated maize grains.

TABLE II.- MEAN NUMBER (\pm SE) OF F₁ ADULTS EMERGED FROM MAIZE GRAINS TREATED WITH DIFFERENT CONCENTRATIONS OF ETHYL L ACETATE EXTRACTS OF PLANT POWDERS.

Treatments	Concentrations (%)					
	0.5	1	1.5	2	2.5	3
<i>Azadirachta indica</i>	42.20 \pm 0.83 g	40.20 \pm 0.83 g	39.80 \pm 1.64 g	34.00 \pm 1.22 g	27.20 \pm 0.83 g	21.20 \pm 0.83 g
<i>Caralluma tuberculata</i>	70.00 \pm 0.22 c	68.60 \pm 1.94 c	65.80 \pm 1.64 c	64.00 \pm 1.58 c	61.60 \pm 1.14 c	59.80 \pm 1.48 c
<i>Allium sativum</i>	56.80 \pm 1.78 e	55.60 \pm 2.30 e	51.60 \pm 1.14 e	49.00 \pm 1 e	45.20 \pm 0.83 e	39.20 \pm 1.48 e
<i>Curcuma longa</i>	47.20 \pm 1.30 f	46.40 \pm 1.51 f	44.20 \pm 0.83 f	42.40 \pm 1.51 f	37.40 \pm 1.14 f	30.00 \pm 2.12 f
<i>Citrullus colocynthis</i>	64.80 \pm 0.83 d	63.20 \pm 1.92 d	60.60 \pm 0.54 d	57.00 \pm 0.70 d	52.80 \pm 0.83 d	48.00 \pm 0.70 d
<i>Calotropis procera</i>	80.80 \pm 0.83 b	76.00 \pm 1.58 b	74.60 \pm 1.67 b	72.40 \pm 1.14 b	72.80 \pm 1.92 b	64.80 \pm 1.48 b
Control	96.00 \pm 1 a	96.00 \pm 1 a	96.00 \pm 1 a	96.00 \pm 1 a	96.00 \pm 1 a	96.00 \pm 1 a
LSD Value	1.50	2.15	1.66	1.55	1.50	1.78

Each value is a mean \pm standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test

Percent infestation of maize grains

The *A. indica* extracts were found most effective in reducing infestation of maize grain as compared to other treatments and control. The number of damaged grains decreased with the increase in the concentration of the plant products (Table III). The number of damaged maize grains were lower across all the plant products treated grains compared to control. The maximum infestation of *S. zeamais* was found in control (39.07%) followed by 19.20% infestation in *C. procera* treated maize grains at 0.5% concentration, contrary to this, the lowest infestation was found in neem seed extracts (4.48), followed by *C. longa* (5.19) extract at 3% concentration.

TABLE III.- EFFECT OF DIFFERENT CONCENTRATIONS OF ETHYL ACETATE EXTRACTS OF PLANT POWDERS ON KERNEL INFESTATION (% \pm SE) OF MAIZE GRAINS BY *S. ZEAMAEIS*.

Treatments	Concentrations (%)					
	0.5	1	1.5	2	2.5	3
<i>Azadirachta indica</i>	10.05 \pm 0.60 e	9.09 \pm 0.21 f	8.17 \pm 0.15 g	7.66 \pm 0.12 g	6.82 \pm 0.31 f	4.42 \pm 0.11 f
<i>Caralluma tuberculata</i>	18.54 \pm 0.42 b	17.93 \pm 0.07 b	16.23 \pm 0.33 c	15.68 \pm 0.57 c	14.47 \pm 0.34 c	14.41 \pm 0.38 b
<i>Allium sativum</i>	15.47 \pm 0.42 c	14.48 \pm 0.25 d	13.40 \pm 0.21 e	12.82 \pm 0.25 e	11.86 \pm 0.14 e	10.34 \pm 0.39 d
<i>Curcuma longa</i>	11.55 \pm 1.17 d	10.25 \pm 0.43 e	9.43 \pm 0.11 f	8.24 \pm 0.16 f	7.16 \pm 0.70 f	6.02 \pm 0.81 e
<i>Citrullus colocynthis</i>	16.24 \pm 0.62 c	15.30 \pm 0.19 c	15.46 \pm 0.53 d	14.96 \pm 0.48 d	13.42 \pm 0.26 d	12.65 \pm 0.85 c
<i>Calotropis procera</i>	19.02 \pm 0.35 b	18.41 \pm 0.05 b	17.61 \pm 0.37 b	16.91 \pm 0.30 b	15.45 \pm 0.94 b	14.44 \pm 0.08 b
Control	40.00 \pm 0.69 a	40.00 \pm 0.69 a	40.00 \pm 0.69 a	40.00 \pm 0.69 a	40.00 \pm 0.69 a	40.00 \pm 0.69 a
LSD Value	0.86	0.58	0.51	0.54	0.72	0.72

Each value is a mean \pm standard error of five replications. Means followed by the same letters along the column are not significantly different at ($P>0.05$) using LSD Test.

Weight loss

The lower weight loss values were recorded in treated maize grains compared to control. The weight loss values for all the treatments increased as the concentration of botanicals decreased. The lowest percent weight loss values (1.93 and 2.35) were recorded in *A. indica* treated maize grains followed by *C. longa* treated maize grains at maximum concentration of 3%. The extracts of *M. azadarach* were found moderately effective among the tested plant products in reducing weight loss of maize grains, in contrary to this, the maximum number of damaged maize grains (12.57 and 7.10%) were noted in *C. procera* and *C. colocynthis* treated maize grains. The efficacy of the tested plant products was dose dependent, the higher the concentration of plant extracts, the higher was the efficacy (Table IV).

Adult longevity

The evaluated plant extracts had significant ($P<0.05$) effect on the adult longevity of the targeted insect. The adult longevity decreased with the increase

in the concentration of the evaluated plant products. Among the tested plant products, the extracts of *A. indica* were found most toxic against the weevils followed by *C. longa* extracts. Among the tested plant extracts, the extracts of *C. procera* and *C. colocynthis* were found least effective against the test insects (Table V).

Sex ratio

It is evident from the recorded data that the application of botanicals had no significant ($P>0.05$) effect on the mean sex ratios of *S. zeamais* when they were reared on treated maize grains. All the evaluated plant products at the tested concentrations had no effect on the sex ratio of the tested insects. A decrease in the number of males was noted compared to females in all the treatments (Table VI).

TABLE IV.- EFFECT OF DIFFERENT CONCENTRATIONS OF ETHYL ACETATE EXTRACTS OF PLANT POWDERS ON WEIGHT LOSS (% \pm SE) OF MAIZE GRAINS BY *S. ZEAMAI*S.

Treatments	Concentrations (%)					
	0.5	1	1.5	2	2.5	3
<i>Azadirachta indica</i>	4.37 \pm 0.37 g	4.09 \pm 0.27 g	2.98 \pm 0.64 g	2.16 \pm 0.19 g	2.01 \pm 0.20 g	1.93 \pm 0.15 g
<i>Caralluma tuberculata</i>	8.34 \pm 0.39 c	8.17 \pm 0.19 c	7.82 \pm 0.24 c	7.43 \pm 0.30c	6.98 \pm 0.18 c	6.31 \pm 0.23 c
<i>Allium sativum</i>	6.26 \pm 0.26 e	5.29 \pm 0.58 e	4.43 \pm 0.12 e	4.27 \pm 0.02 e	4.08 \pm 0.08 e	3.76 \pm 0.37 e
<i>Curcuma longa</i>	5.37 \pm 0.28 f	4.56 \pm 0.27 f	3.83 \pm 0.25 f	3.43 \pm 0.36 f	3.04 \pm 0.11 f	2.35 \pm 0.30 f
<i>Citrullus colocynthis</i>	7.10 \pm 0.25 d	6.92 \pm 0.17 d	5.56 \pm 0.04 d	5.03 \pm 0.02 d	5.00 \pm 0.08 d	4.37 \pm 0.28 d
<i>Calotropis procera</i>	12.57 \pm 0.25 b	11.97 \pm 0.32 b	11.18 \pm 0.39 b	11.10 \pm 0.14 b	10.86 \pm 0.13 b	9.36 \pm 0.29 b
Control	28.64 \pm 0.48 a	28.64 \pm 0.48 a	28.64 \pm 0.48 a	28.64 \pm 0.48 a	28.64 \pm 0.48 a	28.64 \pm 0.48 a
LSD Value	0.44	0.46	0.47	0.35	0.29	0.41

Each value is a mean \pm standard error of five replications. Means followed by the same letters along the column are not significantly different at ($P>0.05$) using LSD Test

TABLE V.- EFFECT OF DIFFERENT CONCENTRATIONS OF ETHYL ACETATE EXTRACTS OF PLANT POWDERS ON ADULT LONGEVITY OF *S. ZEAMAI*S REARED ON MAIZE GRAINS.

Treatments	Concentrations (%)					
	0.5	1	1.5	2	2.5	3
<i>Azadirachta indica</i>	39.60 ± 1.81 e	37.60 ± 1.14 d	35.60 ± 0.89 e	34.00 ± 1 f	33.40 ± 1.14 g	32.20 ± 0.83 e
<i>Caralluma tuberculata</i>	45.00 ± 1 bc	44.80 ± 0.83 b	43.20 ± 0.83 b	42.40 ± 0.54 b	40.60 ± 0.54 c	38.40 ± 0.54 c
<i>Allium sativum</i>	42.60 ± 1.51 d	41.20 ± 0.83 c	40.60 ± 0.89 d	38.80 ± 0.83 d	37.00 ± 0.70 e	35.20 ± 0.83 d
<i>Curcuma longa</i>	41.20 ± 0.83 de	41.80 ± 0.83 c	40.80 ± 0.83 cd	36.80 ± 0.83 e	35.20 ± 0.83 f	34.60 ± 0.89 d
<i>Citrullus colocynthis</i>	43.00 ± 0.70 cd	42.00 ± 1.2 c	41.80 ± 0.83 c	40.40 ± 0.54 c	38.60 ± 0.89 d	37.40 ± 0.54 c
<i>Calotropis procera</i>	46.80 ± 0.83 b	45.40 ± 0.89 b	43.80 ± 0.83 b	43.00 ± 0.70 b	42.00 ± 0.70 b	40.60 ± 0.89 b
Control	49.20 ± 0.83 a	49.20 ± 0.83 a	49.20 ± 0.83 a	49.20 ± 0.83 a	49.20 ± 0.83 a	49.20 ± 0.83 a
LSD Value	2.30	1.23	1.10	1.00	1.07	1.01

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

TABLE VI.- SEX RATIO (MALES/50 FEMALES) OF *S. ZEAMAI*S REARED ON FIVE REPLICATES OF MAIZE GRAINS TREATED WITH DIFFERENT CONCENTRATIONS OF ETHYL ACETATE EXTRACTS OF PLANT POWDERS.

Treatments	Concentrations (%)					
	0.5	1	1.5	2	2.5	3
<i>Azadirachta indica</i>	46.96 ± 0.70 ^{NS}	47.13 ± 1.08 ^{NS}	46.79 ± 1.40 ^{NS}	46.09 ± 1.22 ^{NS}	45.66 ± 1.48 ^{NS}	46.00 ± 1.96 ^{NS}
<i>Caralluma tuberculata</i>	47.26 ± 1.36	47.06 ± 1.38	47.03 ± 1.46	47.30 ± 0.94	46.96 ± 1.90	46.42 ± 1.29
<i>Allium Sativum</i>	47.24 ± 1.90	47.03 ± 1.46	47.13 ± 1.08	48.10 ± 1.37	47.58 ± 0.85	45.62 ± 1.28
<i>Curcuma longa</i>	46.37 ± 1.59	46.79 ± 1.40	45.16 ± 1.05	46.09 ± 1.22	47.60 ± 1.42	45.38 ± 1.41
<i>Citrullus colocynthis</i>	45.84 ± 1.52	45.16 ± 1.05	46.97 ± 1.45	47.59 ± 1.11	47.36 ± 1.37	47.91 ± 1.36
<i>Calotropis procera</i>	47.90 ± 0.70	46.95 ± 1.57	46.95 ± 1.57	46.63 ± 1.47	46.89 ± 1.03	46.58 ± 1.53
Control	46.38 ± 1.36	46.38 ± 1.36	46.38 ± 1.36	46.38 ± 1.36	46.38 ± 1.36	46.38 ± 1.36
LSD Value	1.78	1.74	1.75	1.63	1.77	1.91

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05) using LSD Test.

DISCUSSION

The aromatic plant extracts contain alkaloids, tannin, polyphenols, organic acids, steroids and flavonoids which affect the behavior, growth, development and fertility of several insect species (Abe, 2007; Barbehenn and Constabel, 2011; Barbehenn and Constabel, 2011; Diop *et al.*, 2016; Jebanesan, 2013; Raja, 2012; Vandenborre *et al.*, 2011;). In the current study, laboratory dietary bioassays of six plant extracts had various biological effects on the maize weevil. The longest developmental duration of maize weevil was noted when it was fed on maize grains incorporated with 3% *A. indica* extracts; which proved its efficacy in adversely affecting the focused insect. (Ouko *et al.*, 2017) reported similar findings while studying the bio-efficacy of organic extracts of *A. sativum* against maize weevil. They concluded that different garlic extracts significantly affected the development of F₁ progeny of maize weevil. In our study minimum number of F₁ adult emergence was recorded on maize grains incorporated with highest concentration (3%) of *A. indica* ethyl acetate extracts. Current biological studies revealed that *C. longa* and *A. indica* extracts significantly inhibited the F₁ adult emergence of weevils suggesting that these extracts are toxic to the tested insects. However, their toxicity to the weevils varied with the concentration of the tested extracts.

The efficacy of *A. indica* extracts is equivalent to the efficacy observed by Mamoon-ur-Rashid *et al.* (2013), they investigated the effect of neem derivatives on spotted bollworm *Earias* sp. (Lepidoptera: Noctuidae). They found that *A. indica* extracts had growth inhibiting properties against spotted bollworm when they were reared on diet incorporated with neem derivatives. Similarly, *C. longa* has a well-known history of insecticidal properties against a variety of insect pests. Tavares *et al.* (2016) concluded that ar-turmerone, a compound extracted from *C. longa* rhizomes inhibited the growth of cabbage looper under laboratory and green house conditions. Turmeric acetone, ethanol or petroleum ether extracts applied at 1,000 ppm protected stored wheat from Angoumois grain moth, *Sitotroga cerealella* (Iqbal *et al.*, 2010). Siddiqi *et al.* (2011) reported growth inhibiting and anti-ovipositional properties of *C. longa* extracts against peach fruit fly, *Bactrocera zonata*.

The secondary metabolites produced by plants act as defensives chemicals against insects. The hexane extracts from *Emblica officinalis* and *Ocimum sanctum* at various concentrations possessed various types of biological activities against *Spodoptera litura* Fabricius (Sharma and Bisht, 2008).

These findings corroborate with the earlier findings of (El-Aswad *et al.*, 2004) who evaluated *Khaya vorensis* A. Chev. (Meliaceae) against cotton leafworm, *Spodeptera littoralis* and observed reduction in adult emergence. They further observed that compounds present in *K. vorensis* prolonged the larval and pupal durations of *S. littoralis* compared to control.

Lowest percent infestation and weight losses were observed in maize grains pre-treated with the highest concentration of *A. indica* and *C. longa* extracts; proving the sensitivity of adults to these extracts. Furthermore, our results indicated that the shortest developmental duration, highest incidence and maximum grain damage was observed in untreated maize grains. In past, similar results have been documented by the use of plant products by many scientists against post-harvest stored grain insect pests (Rahman and Talukder, 2006; Tapondjou *et al.*, 2002). Minimum weight loss in cowpea seeds was recorded in grains incorporated with *Percularia daemia* and *Acorus calamus* aqueous extracts at higher concentration of 10% (Jayakumar, 2010).

The minimum adult longevity of maize weevil was recorded in maize grains treated with highest concentration of *A. indica* extracts. In contrast to this, the maximum adult longevity of weevils was recorded on untreated maize grains. The ingestion of cotton leaves treated with 25000 and 30000ppm significantly affected the total longevity of mated and unmated female of cotton mealybug, *Phenacoccus solenopsis* (Mamoon-ur-Rashid *et al.*, 2012). Ouko *et al.* (2017a) also reported that the efficacy of the selected crude extracts was reliant on the dose used, the mortality increased with an increase in the concentration of the extracts. This is similar to the findings of Chaubey (2014) who showed a positive concentration dependent correlation of *A. sativum* oils verses mortality against pulse beetle.

CONCLUSION

Feeding maize weevil on *A. indica* and *C. longa* extracts treated food significantly affected the biology and performance of maize weevil and has potential insecticidal effects signifying their value as eco-friendly bio-pesticides against maize weevil.

ACKNOWLEDGEMENT

The authors thank Pakistan Science Foundation (PSF), Islamabad, Pakistan

for providing financial support for this study under Grant No. PSF/NSLP/KP-GU (424).

REFERENCES

- ABE, I., 2007. Enzymatic synthesis of cyclic triterpenes. *Nat. Prod. Rep.*, **24**: 1311-1331.
- ADARKWAH, C., OBENG-OFORI, D., BÜTTNER, C., REICHMUTH, C. AND SCHÖLLER, M., 2012. Potential of *Lariophagus distinguendus* (Förster) (Hymenoptera: Pteromalidae) to suppress the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in bagged and bulk stored maize. *Biol. Control*, **60**: 175-181.
- ADEBAYO OJO, J. AND AMOS OMOLOYE, A., 2012. Rearing the maize weevil, *Sitophilus zeamais*, on an artificial maize–cassava diet. *J. Insect Sci.*, **12**: 69.
- ADEDIRE, C.O., OBEMBE, O.M., AKINKUROLERE, R.O. AND ODULEYE, S.O., 2011. Response of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. *J. Pl. Dis. Protec.*, **118**(2): 75-79.
- ASHAMO, M.O., ODEYEMI, O.O. AND OGUNGBITE, O.C., 2013. Protection of cowpea, *Vigna unguiculata* L.(Walp.) with *Newbouldia laevis* (Seem.) extracts against infestation by *Callosobruchus maculatus* (Fabricius). *Arch. Phytopathol. Pl. Protec.*, **46**: 1295-1306.
- BARBEHENN, R.V. AND CONSTABEL, C.P., 2011. Tannins in plant–herbivore interactions. *Phytochemistry*, **72**: 1551-1565.
- BOUTARD, A., 2012. *Beautiful corn: America's original grain from seed to plate*. New Society Publishers.
- CHAUBEY, M.K., 2014. Biological activities of *Allium sativum* essential oil against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). **60**: 41. doi:<https://doi.org/10.2478/hepo-2014-0009>.
- CHU, C.-C., SPENCER, J.L., CURZI, M.J., ZAVALA, J.A. AND SEUFFERHELD, M.J., 2013. Gut bacteria facilitate adaptation to crop rotation in the western corn rootworm. *Proc. Nat. Acad. Sci.*, **110**: 11917-11922.
- DE SOUZA TAVARES, W., AKHTAR, Y., GONÇALVES, G.L.P., ZANUNCIO, J.C. AND ISMAN, M.B., 2016. Turmeric powder and its derivatives from *Curcuma longa* rhizomes: Insecticidal effects on cabbage looper and the role of synergists. *Sci. Rep.*, **6**: 34093.
- DIOP, M., SENE, A. AND SAMB, A., 2016. Phytochemical screening and insecticidal activity of three extracts of *Tapinanthus bangwensis* (Engl. Krause) on *Sitophilus zeamais* (Maize Weevil). *Open Access Libr. J.*, **3**: 1.
- DUBEY, N., SHUKLA, R., KUMAR, A., SINGH, P. AND PRAKASH, B., 2010. Prospects of botanical pesticides in sustainable agriculture. *Curr. Sci.*, **98**: 479-480.
- EL-ASWAD, A.F., ABDELGALEIL, S.A. AND NAKATANI, M., 2004. Feeding deterrent and growth inhibitory properties of limonoids from *Khaya senegalensis* against the cotton leafworm, *Spodoptera littoralis*. *Pest Manage. Sci. formerly Pestic. Sci.*, **60**: 199-203.
- HSIAO, T.C., HENG, L., STEDUTO, P., ROJAS-LARA, B., RAES, D. AND FERERES, E., 2009. AquaCrop—the FAO crop model to simulate yield response to water: III. Parameterization and testing for maize. *Agron. J.*, **101**: 448-459.
- IQBAL, J., JILANI, G. AND ASLAM, M., 2010. Growth inhibiting effects of plant extracts against

- the grain moth, *Sitotroga cerealella* (Oliv.) (Gelechiidae: Lepidoptera). *Pakistan. J. Zool.*, **42**: pp.
- JAYAKUMAR, M., 2010. Oviposition deterrent and adult emergence activities of some plant aqueous extracts against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *J. Biopestic.*, **3**: 325.
- JEBANESAN, A., 2013. Secondary metabolites of plants used in pest control. *Int. J. Curr. Mol. Res.*, **1**: 12-15.
- MAMOON-UR-RASHID, M., KHATTAK, M. K., M., AMIR, M., ABDULLAH, K. AND HASHIM, M., 2013. Effect of neem derivatives on spotted bollworm *Earias* sp. (Lepidoptera: Noctuidae) and its egg parasitoid, *Trichogramma chilonis* (Ishi) (Hymenoptera: Trichogrammatidae). *Afr. Ent.*, **21**: 304-309.
- LEKEI, E.E., NGOWI, A.V. AND LONDON, L., 2014. Farmers' knowledge, practices and injuries associated with pesticide exposure in rural farming villages in Tanzania. *BMC Pub Hlth.*, **14**: 389.
- LÓPEZ, M.D., JORDÁN, M.J. AND PASCUAL-VILLALOBOS, M.J., 2008. Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. *J. Stored Prod. Res.*, **44**: 273-278.
- OGUNGBITE, O.C. AND OYENIYI, E.A., 2014. *Newbouldia laevis* (Seem) as an entomocide against *Sitophilus oryzae* and *Sitophilus zeamais* infesting maize grain. *J. biol. Sci.*, **7**: 49-55.
- OKOYE, F.B. AND OSADEBE, P.O., 2009. Studies on the mechanisms of anti-inflammatory activity of the extracts and fractions of *Alchornea floribunda* leaves. *Asian Pacific J. Trop. Med.*, **2**: 7-14.
- OLAYINKA-OLAGUNJU, J., 2014. Effects of two eco-friendly insecticides (*Dennetia tripetala* and *Piper guineense*) against *Dermestes maculatus* (Degeer) on smoked dried *Clarias gariepinus*. *J. environ. Sci. Toxicol. Fd. Technol.*, **8**: 37-43.
- ONI, M. AND ILEKE, K., 2008. Fumigant toxicity of four botanical plant oils on survival, egg laying and progeny development of the dried yam beetle, *Dinoderus porcellus* (Coleoptera: Bostrichidae). *J. agricul. Res.*, **4**: 31-36.
- OPIT, G., PHILLIPS, T.W., AIKINS, M.J. AND HASAN, M., 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. *J. econ. Ent.*, **105**: 1107-1114.
- OUKO, R., KOECH, S., ARIKA, W., NJAGI, S. AND ODUOR, R., 2017a. Bioefficacy of organic extracts of *Ocimum basilicum* against *Sitophilus zeamais*. *Ent. Ornithol. Herpetol.*, **6**: 2161-0983.1000190.
- OUKO, R., KOECH, S., ARIKA, W., OSANO, K. AND OGOLA, P., 2017b. Bioefficacy of organic extracts of *A. sativum* against *S. zeamais* (Coleoptera; Dryophthoridae). *Biol. Syst. Open. Access*, **6**: 2.
- RAHMAN, A. AND TALUKDER, F., 2006. Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus*. *J. Insect Sci.*, **6**.
- RAJA, R.R., 2012. Medicinally potential plants of Labiateae (Lamiaceae) family: an overview. *Res. J. med. Pl.*, **6**: 203-213.
- REES, D.P., 2004. *Insects of stored products*. CSIRO Publishing.
- ROSEGRANT, M.W., MSANGI, S., RINGLER, C., SULSER, T.B., ZHU, T. AND CLINE, S.A.,

2008. *International model for policy analysis of agricultural commodities and trade (IMPACT): Model description*. International Food Policy Research Institute, Washington, DC.
- SHARMA, R. AND BISHT, R., 2008. Antifeedant activity of indigenous plant extracts against *Spodoptera litura* Fabricius. *J. Insect Sci.*, **21**: 56.
- SIDDIQI, A.R., RAFI, A., NAZ, F., MASIHI, R., AHMAD, I. AND JILANI, G., 2011. Effects of *Curcuma longa* extracts on mortality and fecundity of *Bactrocera zonata* (Diptera: Tephritidae). *Ciê. Agrotecnol.*, **35**: 1110-1114.
- STADLINGER, N., MMOCHI, A.J., DOBO, S., GYLLBÄCK, E. AND KUMBLAD, L., 2011. Pesticide use among smallholder rice farmers in Tanzania. *Environ. Dev. Sustain.*, **13**: 641-656.
- STRICEVIC, R., COSIC, M., DJUROVIC, N., PEJIC, B. AND MAKSIMOVIC, L., 2011. Assessment of the FAO AquaCrop model in the simulation of rainfed and supplementally irrigated maize, sugar beet and sunflower. *Agric. Water Manage.*, **98**: 1615-1621.
- SULEIMAN, R., ROSENTRATER, K.A. AND BERN, C., 2015. Evaluation of maize weevils *Sitophilus zeamais* Motschulsky infestation on seven varieties of maize. *J. Stored Prod. Res.*, **64**: 97-102.
- TAPONDJOU, L., ADLER, C., BOUDA, H. AND FONTEM, D., 2002. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *J. Stored Prod. Res.*, **38**: 395-402.
- TEFERA, T., DEMISSIE, G., MUGO, S. AND BEYENE, Y., 2013. Yield and agronomic performance of maize hybrids resistant to the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Crop Protec.*, **46**: 94-99.
- UKEH, D.A., WOODCOCK, C.M., PICKETT, J.A. AND BIRKETT, M.A., 2012. Identification of host kairomones from maize, *Zea mays*, for the maize weevil, *Sitophilus zeamais*. *J. Chem. Ecol.*, **38**: 1402-1409.
- VANDENBORRE, G., SMAGGHE, G. AND VAN DAMME, E.J., 2011. Plant lectins as defense proteins against phytophagous insects. *Phytochemistry*, **72**: 1538-1550.
- ZIBAEE, A., 2011. Botanical insecticides and their effects on insect biochemistry and immunity. In: *Pesticides in the modern World-pests control and pesticides exposure and toxicity Assessment*. InTech.

EVALUATION OF ANTIBIOTIC RESIDUES IN BROILER CHICKEN COLLECTED FROM SOME CITIES OF PUNJAB, PAKISTAN

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Abstract.- The poultry meat is the second most eaten food all over the world and the production of poultry meat has increasing tremendously all over the world. To prevent the diseases and maintain the continuous supply to fulfil the demand of poultry meat for human consumption, antibiotics are used in Pakistan. In this research work, 36 samples of broiler meat (18 muscles and 18 liver samples) were collected from six different cities of Punjab, Pakistan. viz; Islamabad, Faisalabad, Kasur, Multan, Lahore and Bahawalnagar. The antibiotics were detected using HPLC-UV detector. Two antibiotics; levofloxacin and ciprofloxacin were detected from the chicken tissues. The presence of antibiotics in broiler muscles were observed in 33% samples belonging to Islamabad, Faisalabad, Kasur and Bahawalnagar, whereas, in the case of liver both antibiotics were detected from all localities. The antibiotics concentrations in the muscle samples from Lahore and Gujranwala were below detection limits. Highest concentration in the muscle samples were detected in Bahawalpur (levofloxacin 41.95µg/g and ciprofloxacin 23.74µg/g). In case of liver samples, antibiotics were detected in all samples except Kasur. The mean concentration of antibiotics residues in muscles and liver samples were below the permissible limits (200µg/g for liver and 100µg/g for muscles) described by European Union-maximum residual limit (EU-MRL) except the liver sample from Bahawalnagar (ciprofloxacin; 249.86µg/g). The chicken muscles are the major part and preferably consumed by the local community. On the basis of the present results highlighted that broiler meat is safe for human consumption. This study was small scale; however, more research is required to confirm on large scale.

Keywords: Levofloxacin, Ciprofloxacin, Broiler's muscle liver, Poultry.

INTRODUCTION

Poultry sector is one of the most important industries in the worlds, providing protein based food. This industry is growing very fast and is the biggest agriculture based industries throughout the world (Hamra, 2010). Since 1960's the global poultry production has growing faster as compare to any other meat both in developed and developing countries (Chang, 2007). The production of poultry meat has continuously increasing at the rate of 4% on annual basis

worldwide (Nasim *et al.*, 2016). Now a day, poultry farming is a huge business that is divided into various operations like pullet farming for production of meat and farming for egg production and hatcheries (Hamra, 2010).

Poultry industries produce relatively inexpensive and easily available food to people. Commercial level poultry farming also provide employment to people (Farrell, 2012). Chicken meat is of two main types one is broiler and the other one is layer. Meat obtain from boiler farming provide high quality protein to human body whereas protein and vitamin rich eggs are obtained from layer farm (Hamra, 2010). As the human population is increasing rapidly, the industry of poultry has continued to grow in order to meet the demand of human population. Quality of poultry product is very important to ensure good human health.

Productivity of poultry industry has also increased with the increasing demand of meat and eggs. In the world, Pakistan has ranked the 11th biggest poultry producing country with annual production of broiler 1.02 billion (Pakistan economic survey, 2015-2016). Broiler production was 874.09 million in 2015-2016 while it was 722.39 million in 2013-2014 (Pakistan economic survey, 2016).

With the increasing demand of poultry meat there is a need to modify modern facilities, genetic improvement, integrated production system, nutritional practices and management (Gouvãa, *et al.*, 2015). Disease to animals may cause a wide range of socio-economic and biophysical effects that may localize or global or may be direct or indirect (Perry and Sones, 2009). In the past few decades, there have been generally decrease in livestock disease because of more effective vaccines and medical drugs and improvement in diagnostic services and technologies (Thornton, 2010). In future, threats of infectious diseases will continue dynamic, combating and diverse development of completely unpredicted disease will require a detection system that is adaptable and flexible in all face of change (King *et al.*, 2006).

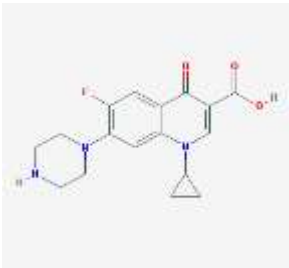

Presently antibiotics are used for anticipation of disease, improving the growth and efficacy of feeding are crucial to energetic animals farming business, use of AGP has increasing progressively (Doyle, 2001). There is a growing concern that antimicrobial use in veterinary compromises the health of human, with bacterial resistance in animals and spread to human via food from animals and spreading to environment (Castanon, 2007). When these antibiotics are given to poultry birds they become a part of their body and then transfer from meat to

human that feed on them poses health impact (Marshall & Levy, 2011). Unlawful use of antibiotics, before slaughtering, untimely drawing time period and not to follow the labeled instructions leads to adulteration of poultry meat with antibiotic residues that are further use for food, ultimately cause potential adverse effect to human health (Donoghue, 2003). WHO has suggested that the antibiotics that are also certified for human medicines must not be used as growth promoters in poultry production (Singh *et al.*, 2014).

Quinolones actions are based on anti-DNA activities. Quinolones are widely use in veterinary as well as human medicine because of its broad spectrum antibacterial activity and good tolerance (Sharma *et al.*, 2009). DNA gyrase catalytic reaction in bacterial DNA that doubles the strand chain, allow separation, relaxation, supercoiling, and re-integration through translation and transcription (Gouvãa *et al.*, 2015). In 1980s flourquinolones were introduced and it is fluorinated derivative of quinolones as it has flourne attached at 6th position. They also have a keton in 4th position and carboxyl in 3rd position, providind additional antibacterial action spectrum to flourquinolones (Sharma *et al.*, 2009). Flourquinolones have tendency to rapidly absorb after administration. As little binding to plasma proteins and wide distribution volume are eliminated by bile and urine and their remains can be found in the kidneys and liver (Goetting *et al.*, 2011).

Ciprofloxacin is the metabolite of Enrofloxacin. It has different pharmacokinetics and bioavailability is half in the bird's body as compared with enrofloxacin (Ito *et al.*, 2005). Ciprofloxacin and Enrofloxacin are second generation flouroquinolones have broad spectrum antimicrobial activity. After oral administration, both have good bioavailability to tissue distribution (Papich, 1998). The most popular and most widely used fluoroquinolone, that is ciprofloxacin was promoted in 1986 and since then the value of fluoroquinolones have become widely recognized for the treatment of wide range of infections (Sharma *et al.*, 2009). Levofloxacin is a broad spectrum antibiotic that is active against gram-positive and gram-negative bacteria. Levofloxacin is excreted as unchanged drug after the oral administration (Kumar Shahwal *et al.*, 2013). It is a newer third generation fluoroquinolone (Patel *et al.*, 2009). Levofloxacin is well tolerated generally and has a good penetration capacity in tissue and adequate concentration can be maintained at the infection site (Noreddin and Elkhatib, 2010).

TABLE I.- CHEMICAL STRUCTURES OF CIPROFLOXACIN AND LEVOFLOXACIN

Antibiotics class	Chemical structure	Mode of action	Activity spectrum
Ciprofloxacin		Inhibite DNA gyrase	Gram +ve, -ve bacteria
Levofloxacin		Inhibite DNA gyrase	Gram +ve, -ve bacteria

Source : (Naeem *et al.*, 2006).

MATERIALS AND METHODS

Chemicals and reagents

Levofloxacin hemihydrate (96.95%) and Ciprofloxacin (99.31%), Meta-phosphoric acid, sodium dodecyle-sulphate, HPLC grade acetonitrile were purchased from sigma Aldrick. n-hexane, sodium dihydrogen-phosphate, ethanol, HPLC grade methanol and phosphoric acid and Double Distilled water were purchased from Merck Germany.

Samples collection and storage

The samples were collected from six major cities of Punjab, Pakistan as Islamabad, Lahore, Faisalabad, Kasur, Gujranwala and Bahawalnagar. Samples was collected from three different sites of these six cities to conduct the

representative study and observed spatial difference of antibiotics used among these cities.

Sample size of 36 broiler chicken was collected. Samples consisted of (n=18) broiler thigh muscles and (n=18) broiler liver sample. Samples were collected from six different cities of Punjab, Pakistan. Samples were packed in self-sealing transparent polythene bags and immediately placed in ice box having temperature 20°C during transportation. Then samples were stored at 4°C in freezer upon arrival in laboratory till the analysis had started.

Standard solutions preparation

Standard solutions of antibiotics like levofloxacin hemihydrate and ciprofloxacin were prepared. The standard stock solutions were prepared by mixing them in methanol as 1000µg/1ml (10mg in 10 ml) and can be stored at 4°C but no longer than two months. Further standard working dilution were prepared from the stock solution and made volume up to the mark with methanol in volumetric flask. Working dilutions were made fresh every time.

Processing of extracting and deflating and cleanup

The frozen samples (muscle and liver) were taken and thawed first at room temperature till no longer ice crystals had remain in the sample. The thawed samples were chopped separately (thigh and liver) with the help of ordinary kitchen chopper properly for 4-6 min. These chopped samples were taken in properly cleaned and sterilized petri plates with great care and covered with lid.

Chopped samples (thigh and liver) of 10 ± 0.5 g weight were taken into a flask; carefully weigh with the help of analytical weighing balance and spatula. The deproteinization was done with the addition of 30ml of meta-phosphoric acid (0.3%): acetonitrile (1:10) with pH of 4.1 (Su *et al.*, 2003). It was homogenized for 30-35 minute on orbital mechanical shaker at high speed of 250rpm. The extract was filtered with the help of Buchner funnel to avoid the blockage of C18 cartridge. The Buchner funnel assembly was properly cleaned before use to avoid any contamination. The filtrate was transferred into cleaned flask with sufficient care.

Defatting was done by adding 25ml of n-hexane in the extract and then shaken it well on orbital mechanical shaker for 30 mint at 200 rpm. By using

cleaned separating funnel, these two layers were separated. Bottom layer was collected and the process of defatting was done again for complete defatting. The bottom layer as extract was taken in a round bottom flask and evaporated till complete dryness with the help of rotary evaporator at the temperature of 50°C with 160rpm.

The residues were dissolved in 10ml of methanol. Cartridge was activated with 5ml of methanol and rinse with 10ml distilled water with the flow rate of 1ml per mint. Dissolved residues were loaded to bond elute C18 cartridge. The concentration bottle were washed twice with 5ml of methanol and loaded to cartridge for elution at flow rate of 1ml per mint. The eluent were collected and evaporated by rotary evaporator at the temperature of below 60°C with 160rpm. The residues after evaporation were dissolved in 1ml of mobile phase. This was then collected in an eppendorf tube. Extracts were then membrane filtered with the help of syringe filter having 0.45µm pore size filter paper to avoid any blockage in HPLC capillary.

HPLC

Mobile phase was prepared by mixing acetonitrile and 0.05M NaH₂PO₄ in the ratio of (35:65 v/v) and 3.5mM sodium dodecyl-sulphate were added. The pH of NaH₂PO₄ was adjusted to 2.5 (Su *et al.*, 2003). Mobile phase was filtered by membrane filtration, prior to use. Sonicator was used for degassing of mobile phase. Sonication was done for 15mint at ambient temperature. Mobile phase for HPLC was made daily.

An isocratic high performance liquid chromatography system with UV-Vis detector having scan range (190-700nm), separation was done with Reverse phase C18 column, 4.6 ID×250mm length was used. The wavelength was set at 260nm and column temperature was adjusted to 25±5°C. Mobile phase was acetonitrile: 0.05 M NaH₂PO₄ pH=2.5 (35:65 v/v) + 3.5mM sodium dodecyl-sulphate and 1ml/min flow rate was set. Injection volume of sample was 20µl, liquid-liquid extraction process was used.

Detection and quantification

Levofloxacin and ciprofloxacin were identified from the samples by comparing the retention time and area under the curve of unknown substance with the standards (Ciprofloxacin and levofloxacin). First the peaks of individual

standards were taken. Then mixed standards solution was analyzed with HPLC. Quantitative analysis was calculated by using following equation.

$$X(\mu\text{g/kg}) = \frac{A}{B} \times 100$$

X is amount of each quinolone in sample, A is area covered by sample peak, and B is area covered by Standard peak

Statistical analysis

Excel 2013 and SPSS softwares were used to organize the data, making graphs, tables and to perform statistical analysis to data. Descriptive stats were performs on the results of concentration of antibiotics from samples. Multivariate test of significance was applied to find the statistical Significant on distribution of antibiotics concentration among broiler's organ and different localities (p-value \leq 0.05). ArcGIS 10.3 was also used to show the sampling location.

RESULTS AND DISCUSSION

Samples of broiler's livers and thigh muscles from six different cities of Punjab, Pakistan were analyzed for the determination of residual level of selected antibiotics. Figure 1 shows the sampling locations from six different cities of Punjab, Pakistan.

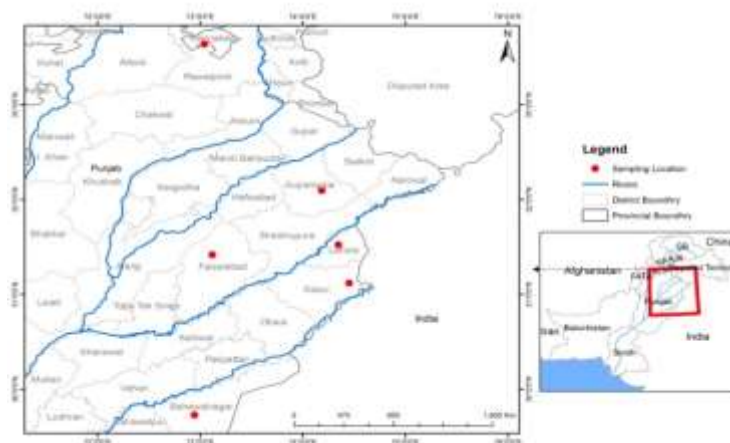


Fig. 1. Map of Punjab showing sampling locations

Analytical conditions of HPLC

The pH factor of mobile phase for HPLC was a main factor in achieving chromatographic separation of quinolones. This was also noted by many authors Gigosos *et al.*, 2000; Ramos *et al.*, 2003; Schneider *et al.*, 2007. As pH is a power tool for the optimization of separation of analytic mixture and column deliver the same peak shape at a broad pH range from (2.0 to 7.0) and it might be useful to achieve solvent optimization (Ramos *et al.*, 2003). Fluoroquinolones do not fluorescence strongly at the pH of 10 and do at pH 3-4 (Schneider *et al.*, 2007). For HPLC analysis of polar and basic substances, a problem might be face as severe peak broadening and tailing on reverse phase column. The ampholytic compounds may cause tailing peak in general reverse phase chromatography. Metal impurities and residual sylanol groups in traditional phase column are major to cause this tailing in reverse phase chromatography that make it difficult in achieving separation by chromatography that is suitable for the analysis of residues in order to fulfill the requirement as adequate resolution, peak shapes and short retention time. This behavior can be minimized by mobile phase that has high ionic strength and acidity and using ion pairing techniques. Different concentration of sodium dodecyl-sulphate in the mobile phase can cause quite different peak shapes in the column (Ramos *et al.*, 2003). Mobile phase for this research work was selected of low acidic pH. The mobile phase was acetonitrile and 0.05M NaH₂PO₄ in the ratio of (35:65 v/v) and 3.5mM sodium dodecyl-sulphate as by Su *et al.* (2003).

According to Horie *et al.* (1995) study on various concentration of sodium dodecyl- sulphate ranging from 2-5 mM were added to acetonitrile: 0.05M NaH₂PO₄ (pH 2.5) in (35/65 v/v) to study the effect on separation of quinolones. As hydrophobic nature of first generation of quinolones, they are neutral compounds in acidic medium therefore they are not affected by ion-pairing reagents. Second generation quinolones have two ionizable group as Piperazine and carboxylic acid so they are cation in acidic medium, having strong polarity. Their results showed that 3.5mM sodium dodecyl sulphate has better separation effects.

Sample selection

The present research work was conducted for the determination of antibiotics residues in the broiler chicken collected from local market of different cities of Punjab, Pakistan. The antibiotics residue that was detected includes

ciprofloxacin and levofloxacin. The decision about the selection of broiler's tissues was based on the main edible part of muscle and the main metabolizing organ. So Broilers tissues that was selected for this research work was liver and thigh muscle. The level of antibiotics residues may vary in different tissues. Therefore, two different tissues were selected in order to check the relative distribution of these antibiotics among them. There remains a choice in selecting the muscle either thigh muscle or breast muscle. In previous study more antibiotics residues were detected from thigh muscle samples as compared to the breast muscle samples (Attari *et al.*, 2014). So thigh muscle was selected for the present research work.

Confirmation of antibiotic residues

In the present study the residues of ciprofloxacin and levofloxacin was detected in broilers liver and muscle by high performance liquid chromatography HPLC-UV method validated by (Su *et al.*, 2003). According to the survey of antibiotics residues among those cities, Bahawalnagar showed 100% positive muscle samples whereas Faisalabad, Bahawalnagar, Kasur and Gujranwala showed 100% positive liver samples The results of ciprofloxacin of present study were in line with the study of Omotoso and Omojola (2014) and also in line for the results of levofloxacin Kyuchukova *et al.* (2013). They investigate there samples for the presence of fluoroquinolones by HPLC-UV method.

Antibiotics residues in thigh muscles and liver

In the present study, the thigh muscle and liver samples collected different cities of Punjab Pakistan, were detected for levofloxacin and ciprofloxacin concentration by HPLC-UV. The antibiotics concentrations in the muscle samples from Lahore and gujranwala were below detection limits. While Bahawalnagar's thigh muscle sample showed highest mean concentration of levofloxacin ($41.95 \mu\text{gkg}^{-1}$) and ciprofloxacin ($23.74 \mu\text{gkg}^{-1}$) residues in thigh samples. This was may be due to excessive use antibiotics in Bahawalnagar. The concentration of levofloxacin and ciprofloxacin of present study was compared to the European Union maximum residual limit (MRL), no sample exceeded MRL limit. Mean concentration of antibiotics residues in thigh muscle samples among different cities of Punjab Pakistan is shown in Figure 2.

In case of liver samples, Bahawalnagar samples shown highest mean concentration of levofloxacin and ciprofloxacin as compared to the other

samples. One of the bahawalnagar sample exceed from the permissible limite described by EU MRL, as levofloxacin ($159.27\mu\text{gkg}^{-1}$) and ciprofloxacin ($249.86\mu\text{gkg}^{-1}$). This was might be due to excessive use of antibiotics in Bahawalnagar. Mean concentration of antibiotics residues in broiler's liver samples from different cities of Punjab Pakistan is shown in Figure 3.

TABLE II. SURVEY OF ANTIBIOTICS RESIDUES IN SAMPLES.

Sampling cities	Muscle samples		Liver samples		Muscle samples exceed EUMRL %	Liver samples exceed EUMRL %	EU MRL $\mu\text{g/kg}$	
	Positive %	Negative %	Positive %	Negative %			Muscles	Liver
Lahore	0	100	66.7	33.3	0	0	100	200
Islamabad	33.3	66.7	66.7	33.3	0	0	100	200
Faisalabad	33.3	66.7	100	0	0	0	100	200
Bahawalnagar	100	0	100	0	0	33.3	100	200
Kasur	33.3	66.7	100	0	0	0	100	200
Gujranwala	0	100	100	0	0	0	100	200

* EUMRL= European Union Maximum Residual Limit

TABLE III.- SPATIAL VARIATION OF ANTIBIOTICS RESIDUES IN THIGH AND LIVER SAMPLES.

Sampling cities	Broiler's thigh muscles samples				Broiler's liver samples			
	Levofloxacin		Ciprofloxacin		Levofloxacin		Ciprofloxacin	
	Range (μgkg^{-1})	Mean \pm SD (μgkg^{-1})	Range (μgkg^{-1})	Mean \pm SD (μgkg^{-1})	Range (μgkg^{-1})	Mean \pm SD (μgkg^{-1})	Range (μgkg^{-1})	Mean \pm SD (μgkg^{-1})
Lahore	ND	ND	ND	ND	0-0.25	0.08 \pm 0.14	0-3.89	1.29 \pm 2.24
Islamabad	0-34.98	11.66 \pm 20.19	0-31.36	10.45 \pm 18.10	0-9.54	4.38 \pm 4.81	0-3.00	1.53 \pm 1.505
Faisalabad	ND	ND	0-7.67	2.55 \pm 4.42	0-0.02	0.006 \pm 0.01	0.3-0.55	0.43 \pm 0.125
Bahawalnagar	8.01-96.86	41.95 \pm 47.99	0-56.28	23.74 \pm 29.15	0.75-159.27	73.35 \pm 80.09	0.35-249.86	89.19 \pm 139.39
Kasur	ND	ND	0-2.19	0.73 \pm 1.26	ND	ND	10.44-12.61	11.21 \pm 1.20
Gujranwala	ND	ND	ND	ND	5.51-33.29	15.14 \pm 15.72	9.27-19.10	15.07 \pm 5.15

*ND= Not Detected

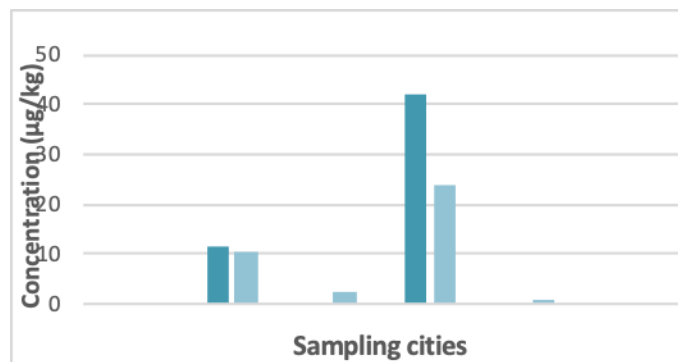


Fig. 2. Mean concentration of antibiotics residues in thigh muscles which cities are these?

In this study the results demonstrated that concentration of levofloxacin and ciprofloxacin residues in liver was higher than that in muscle, these results can also be confirm by several other studies showing the concentration of many drugs are higher in the liver than in muscle of broiler. Mean \pm SD of levofloxacin and ciprofloxacin in muscle was $8.93 \pm 23.85 \mu\text{gkg}^{-1}$ and $6.24 \pm 14.82 \mu\text{gkg}^{-1}$, respectively whereas it was $15.49 \pm 39.06 \mu\text{gkg}^{-1}$ and $19.79 \pm 57.81 \mu\text{gkg}^{-1}$ respectively in liver. This was an evidence of higher concentration of drugs residues in liver as compare to the muscle. The drug residues concentration was higher in liver since liver is the main metabolizing and detoxifying organ. Metabolism of drugs takes place in liver that's why liver interact with several types of xenobiotic and drugs. Moreover it is extremely perfused organ then the muscle. Because of this, the drugs concentration was found greater in liver then in muscle (Nasim *et al.*, 2016). These results were also in with the study of Ammar *et al.* (2016); Kyuchukova *et al.* (2013) Farheen (2012) Amjad *et al.* (2006) and Naeem *et al.*, (2006). There studies showed that the antibiotics residues were higher in liver as compare to the other tissues.

Ciprofloxacin is the well absorbed in gastrointestinal (GI) tract with the protein binding serum of about 20-40% when administered orally (Khan *et al.*, 2015). Ciprofloxacin has been found to be absorbed mainly in the jejunum and duodenum after it is administered orally (Wolfson and Hooper, 1991). Ciprofloxacin is effective for the complicated urinary tract infections (Croom and Goa, 2003). Ciprofloxacin's protein bindings capacity, leads the increasing withdrawal or removal period up to 23 days that is actually 12 to 15 days in usual consideration (Khan *et al.*, 2015). Levofloxacin has plasma protein binding serum is about 24 to 38% (Fish and Chow, 1997). Levofloxacin was predicted to

have withdrawal time period before slaughter is 4 to 5 days. The slower elimination of levofloxacin from the body could be explained as its lipophilicity and high tissue perfusion rate (Ravikumar *et al.*, 2016).

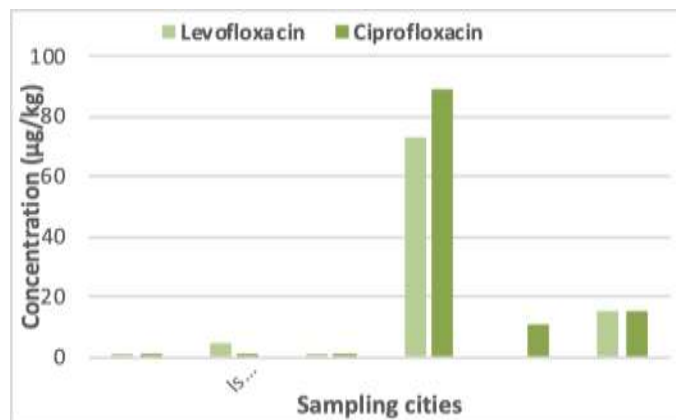


Fig. 3. Mean concentration of antibiotics residues in broiler's liver samples which cities?

According to the results there were a significant difference in the locality as ($p=0.02$). That means the localities had a significant effect on the concentration of levofloxacin and ciprofloxacin. Concentrations of antibiotics residue on locality basis also have significantly affect the human health.

Whereas on organ basis as other independent variable results showed p -value=0.468 which indicate, there is no significant difference in the concentration of drugs residues among organs. It can be relate that there was no significant effect of their concentration on human health.

Pattern of drug usage

Six questionnaires were filled from local boiler growers from sampling cities. Questionnaire was based on open ended questions to analyze whether they were using antibiotics and other such questions. The questionnaire was mainly about the drugs in breeding and frequency of use. They claimed that, they were using these drugs in order to prevent any microbial infection that can cause infection to whole flock. This practices by growers causes negatively effects on bird's health as again and again use of drugs cause resistance to drugs and cause the prevalence drug resistant bacteria in the animals (Geidam *et al.*, 2012). The

drugs residue in meat oriented from animals may transfer to human that consume it, pose adverse health effects in human (Doyle, 2006). According to them levofloxacin, tylosin, doxycycline, amoxycycline, colistin, lincomycin, gentamycin, trimethoprim, ciprofloxacin, chloramphenicol, erythromycin, neomycin, suprofloxacina and norfloxacin are mostly used among these six cities of Punjab, Pakistan. Presently, antibiotics are gaining more importance all over the world. These antibiotics are used not only to cure infection but also used for prevention of infections and promote the growth in production of poultry meat (Phillips *et al.*, 2004).

CONCLUSION AND RECOMMENDATION

Results of this study indicated the presence of antibiotics in local market sold meat. The uses of contaminated food cause resistance in the consumer and cause threats to public health. The concentration of levofloxacin and ciprofloxacin in the chicken liver and thigh muscle sample were compared with the maximum residual limit. No muscle sample was exceeded from MRL value but just one liver sample was exceeded from EU MRL permissible limit. But only Bhawalnagar sample show the highest concentration for levofloxacin and ciprofloxacin. During the present study, sample size is small and there is need to replicate it on large scale to highlight the antibiotic contamination in broiler chicken.

REFERENCES

- AMJAD, H., IQBAL, J. AND NAEEM, M., 2006. Estimation of selected residual antibiotics in muscle, kidney, liver, and egg of layer chicken. *Proc. Pakistan Acad. Sci.*, **43**: 29.
- ATTARI, V. E., ABBASI, M. M., ABEDIMANESH, N., OSTADRAHIMI, A. AND GORBANI, A., 2014. Investigation of enrofloxacin and chloramphenicol residues in broiler chickens carcasses collected from local markets of tabriz, northwestern Iran. *Hlth. Promo. Perspect.*, **4**: 151.
- CASTANON, J., 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult. Sci.*, **86**: 2466-2471.
- CHANG, H. S., 2007. Overview of the world broiler industry: Implications for the Philippines. *Asian J. Agric. Develop.*, **4**: 67-82.
- Commission regulation (EU) No 37/2010 of 22 December 2009 on Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Available at http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-5/reg_2010_37/reg_2010_37_en.pdf, retrieved on 2nd March 2017
- CROOM, K. F. AND GOA, K. L., 2003. Levofloxacin. *Drugs*, **63**: 2769-2802.
- DONOGHUE, D. J., 2003. Antibiotic residues in poultry tissues and eggs: human health concerns? *Poult. Sci.*, **82**: 618-621.

- DOYLE, M. E., 2001. *Alternatives to antibiotic use for growth promotion in animal husbandry*: Food Research Institute, Department Food Microbiology & Toxicology, University of Wisconsin, Madison.
- DOYLE, M. E., BILL, F. AND COFFEE, P., 2001. Alternatives to antibiotic use for growth promotion in animal husbandry. **202**: 222-0749.
- FARHEEN, A., 2012. *Antibiotic residue in poultry products*. University Of Agriculture, Faisalabad.
- FARRELL, D., 2012. The role of poultry in human nutrition. **2**: 2.
- FISH, D. N. AND CHOW, A. T., 1997. The clinical pharmacokinetics of levofloxacin. *Clin. Pharmacok.*, **32**: 101-119.
- GEIDAM, Y. A., ZAKARIA, Z., AZIZ, S. A., BEJO, S. K., ABU, J. AND OMAR, S., 2012. High prevalence of multi-drug resistant bacteria in selected poultry farms in Selangor, Malaysia. *Asian J. Anim. Vet. Adv.*, **7**: 891-897.
- GIGOSOS, P., REVESADO, P., CADAHÄ±A, O., FENTE, C., VAZQUEZ, B., FRANCO, C., 2000. Determination of quinolones in animal tissues and eggs by high-performance liquid chromatography with photodiode-array detection. *J. Chromatogr.*, **871**: 31-36.
- GOETTING, V., LEE, K. AND TELL, L., 2011. Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature. *J. Vet. Pharmacol. Therap.*, **34**: 521-556.
- GOUVÄ±A, R., DOS SANTOS, F. AND DE AQUINO, M., 2015. Fluoroquinolones in industrial poultry production, bacterial resistance and food residues: a review. *Rev. Brasil. CiÄ±ncia AvÄ±cola*, **17**: 1-10.
- HAMRA, C., 2010. *An assessment of the potential profitability of poultry farms: A broiler farm feasibility case study*. Univ. of Tennessee at Martin.
- Horie, M., Saito, K., Nose, N., and Nakazawa, H., 1995. Simultaneous determination of eight quinolone antibacterials in meat and fish by high performance liquid chromatography. *Fd. Hyg. Safe. Sci.*, **36**: 62-67_61.
- ITO, N., MYIYAJI, C., LIMA, E. AND OKABAYASHI, S., 2005. Antimicrobianos: Usos preventivos e curativos em avicultura. *Farmacol. Apl. Avicul. SÄ±o Paulo, Roca*, 115-147.
- KHAN, G. J., KHAN, R. A., MAJEED, I. AND SIDDIQUI, F. A., 2015. Ciprofloxacin; the frequent use in poultry and its consequences on human health. *Profess. Med. J.*, **22**.
- KING, D., PECKHAM, C., WAAGE, J., BROWNLIE, J. AND WOOLHOUSE, M. E., 2006. *Infectious diseases: preparing for the future*. American Association for the Advancement of Science.
- KUMAR SHAHWAL, V., DUBEY, B. K. AND BHOUMICK, M., 2013. Preformulation study of levofloxacin. *Int. J. Adv. Pharmaceut.*, **1**: 01-08.
- KYUCHUKOVA, R., URUMOVA, V., LYUTSKANOV, M., PETROV, V. AND PAVLOV, A., 2013. levofloxacin residues in chicken meat and giblets. *Bulgarian J. Vet. Med.*, **16**: 216-219.
- MARSHALL, B. M. AND LEVY, S. B., 2011. Food animals and antimicrobials: impacts on human health. *Clin. Microbiol. Rev.*, **24**: 718-733.
- NAEEM, M., KHAN, K. AND RAFIQ, S., 2006. Determination of residues of quinolones in poultry products by high pressure liquid chromatography. *J. appl. Sci.*, **6**: 373-379.
- NASIM, A., ASLAM, B., JAVED, I., ALI, A., MUHAMMAD, F. AND RAZA, A., 2016. Determination of florfenicol residues in broiler meat and liver samples using RPÄ± HPLC with UVÄ± visible detection. *J. Sci. Fd. Agric.*, **96**: 1284-1288.

- NOREDDIN, A. M. AND ELKHATIB, W. F., 2010. Levofloxacin in the treatment of community-acquired pneumonia. *Expert Rev. Anti-infect. Ther.*, **8**: 505-514.
- OMOTOSO, A. B. AND OMOJOLA, B. A., 2014. Screening of fluoroquinolone residues in imported and locally produced broiler chicken meat in Ibadan, Nigeria. *Int. J. Hlth. Anim. Sci. Fd. Safe.*, **1**. Pages
- PAKISTAN ECONOMIC SURVEY, 2015-2016, Agriculture Ministry of Finance. Available at <http://www.irispujab.gov.pk/Economic%20Surveys-New/Economic%20Survey%202015-16.pdf>, retrieved on 4th march, 2017.
- PAPICH, M. G., 1998. Antibacterial drug therapy: focus on new drugs. *Vet. Clin. Small Anim. Pract.*, **28**: 215-231.
- PATEL, J. H., VARIA, R. D., PATEL, U. D., VIHOL, P. D., BHAVSAR, S. K. AND THAKER, A. M. 2009. Safety level of levofloxacin following repeated oral administration in White Leg Horn layer birds. *Vet. World*, **2**: 137-139.
- PERRY, B. AND SONES, K., 2009. *Global livestock disease dynamics over the last quarter century: drivers, impacts and implications*. FAO, Rome, Italy. (Background paper for the SOFA 2009).
- PHILLIPS, I., CASEWELL, M., COX, T., DE GROOT, B., FRIIS, C. AND JONES, R., 2004. Does the use of antibiotics in food animals pose a risk to human health? A reply to critics. *J. Antimicrob. Chemother.*, **54**: 276-278.
- RAMOS, M., ARANDA, A., GARCIA, E., REUVERS, T. AND HOOGHUIS, H., 2003. Simple and sensitive determination of five quinolones in food by liquid chromatography with fluorescence detection. *J. Chromatogr. B.*, **789**: 373-381.
- RAVIKUMAR, C., SANGANAL, J., SHRIDHAR, N., NARAYANASWAMY, H., ANSAR, K. AND RAMACHANDRA, S., 2016. Determination of withdrawal period of levofloxacin in liver and breast muscle of dual purpose chicken. *North-East Veter*, **15**: 23-26.
- SCHNEIDER, M. J., BRADEN, S. E., REYES-HERRERA, I. AND DONOGHUE, D. J., 2007. Simultaneous determination of fluoroquinolones and tetracyclines in chicken muscle using HPLC with fluorescence detection. *J. Chromatogr. B.*, **846**: 8-13.
- SHARMA, P. C., JAIN, A. AND JAIN, S., 2009. Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects. *Acta Pol. Pharm.*, **66**: 587-604.
- SINGH, S., SHUKLA, S., TANDIA, N., KUMAR, N. AND PALIWAL, R., 2014. Antibiotic residues: a global challenge. *Phar. Sci. Mon.*, **5**. Pages.
- SU, S. C., CHANG, M., CHANG, C. L., CHANG, P. C. AND CHOU, S.-S., 2003. Simultaneous determination of quinolones in livestock and marine products by high performance liquid chromatography. *J. Fd. Drug Analy.*, **11**: 114-127.
- THORNTON, P. K., 2010. Livestock production: recent trends, future prospects. *Phil. Trans. R. Soc. B: Biol. Sci.*, **365**: 2853-2867.
- WOLFSON, J. AND HOOPER, D., 1991. Pharmacokinetics of quinolones: newer aspects. *Eur. J. Clin. Microbiol. Infect. Dis.*, **10**: 267-274.

**HOW DICHLOROMETHANE AND METHANOL NEEM SEED EXTRACTS
AFFECT COTTON MEALYBUG (*PHENACOCCLUS SOLENOPSIS*)
MORTALITY AND EMERGENCE OF AN ENCYRTID PARASITOID
(*AENASIUS BAMBAWALEI*) UNDER LABORATORY CONDITIONS**

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Abstract.- The toxic effects of Neem seed extracts in methanol and dichloromethane were studied under laboratory conditions against the cotton mealybug, *Phenacoccus solenopsis* Tinsley. The experiment was laid out under Completely Randomized Design (CRD) with two main treatments (Neem seed extracts in methanol and dichloromethane) each with 3 different concentrations and a control, replicated five times. Results showed that among all treatments/concentrations the highest percent mean mortality of all three stages *i.e.* 1st, 2nd instars and adult was recorded in 3% concentration of methanol neem seed extract. In all these treatments, significant mortality ($p > 0.05$) was recorded during the whole weeks as compared to control ($14 \pm 1.87\%$), however dichloromethane extracts showed non-significant ($p < 0.05$) results with each other but were significantly different from control (15 ± 1.58). Similarly all these treatments were checked for compatibility with parasitoid *Aenasius bambawalei* Hayat, which showed non-significant effect on emergence (%) from the pupae. It is concluded that the mortality is directly correlated with dose and time. All treatments showed control but 3% methanol neem seed extract was significantly different from others treatments and therefore the concentration is recommended for management of *P. solenopsis*. However, further study needs to be conducted on field level to confirm these experiments.

Keywords: *Phenacoccus solenopsis*, Neem seed extracts, Methanol, Dichloromethane and *Aenasius bambawalei*

INTRODUCTION

Mealy bugs are soft-bodied, sap-feeding insects with mouthparts adapted to piercing and sucking and they secrete a powdery, white wax covering over the body (Osborne, 1994). They produce a large amount of honeydew which is responsible for the development of a black fungus commonly known as sooty mold (Gullan and Kosztarab, 1997).

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It was found to attack a large number of plant species including crops, vegetables, ornamental plants and weeds (Arif *et al.*, 2009; Abbas *et al.*, 2010). It has many alternative hosts *viz.*, sunflower, vegetables, weeds, ornamentals etc. (Saini *et al.*, 2009). Being a polyphagous pest, the *P. solenopsis* has been recorded to feed on a number of cultivated crops including weeds (Patel *et al.*, 2009).

Phenacoccus solenopsis has a wider geographical distribution with its origin in Central America (Williams and Granara de Willink, 1992). It was described as a serious cum invasive pest of cotton in Pakistan and India (Hodgson *et al.*, 2008) and on *Hibiscus rosa-sinensis* in Nigeria (Akintola and Ande, 2008). Latest report on the invasiveness of *P. solenopsis* has been from the Eastern region of Sri Lanka (Prishanthini and Laxmi, 2009) on ornamentals, vegetable crops and weeds, and in China (Wang *et al.*, 2009) on cotton. *P. solenopsis* appeared on cotton in Pakistan during 2005 and attained pest status in cotton growing areas of Punjab and Sind provinces.

Integrated pest management of mealy bug could be the safest and cheapest method of pest control (Ahmad *et al.*, 2011). However, the use of insecticides is inevitable to check the mealy bug outbreaks as compared to predators and parasitoids (McKenzie *et al.*, 2010). Several insecticides belonging to different groups have been documented as effective against cotton mealybug. For example, Suresh *et al.* (2010) recommended a need based application of insecticides like profenofos 50 EC 2 mL/L, chlorpyrifos 20 EC 2 mL/L, dimethaote 2 mL/L, imidacloprid 0.6 mL/L and thiamthoxam 0.6 g/L. Other insecticidal solutions like buprofezin against nymphal and adult population of bunch infestation (Muthukrishnan *et al.*, 2005) besides insect growth regulators and nicotine based insecticides in some vineyards. Some other non-insecticidal chemical control measures have also been documented and showed useful results (JainHua, 2003).

More than 1000 species of plants have been reported to have chemicals in leaves, stem, flowers, seeds and roots which have insecticidal property, only a few of them have been used for practical insect control on commercial scale in the past. The chemical poisons of plants are mostly alkaloids. Alkaloids are plant products, which are nitrogenous in nature. They are heterocyclic compounds having strong effect on the nervous system of animals. The alkaloidal extracts when applied to the insects bring about disturbance in the nervous system and cause death. They are, therefore, basically nerve poisons (Shahid, 1999).

The neem tree (*Azadirachta indica*, Juss) tree containing bioactive ingredients and more than 100 chemical compounds have been identified so far, but the most effective bio active compounds are azadiractin and salamin. The bioactive compounds are bitter tasting and have the ability to precipitate plant and animal proteins which are considered as defense compounds against organisms, azadrachtin has repellent and insecticidal action against most insects (Schmutterer, 1990). Neem derivatives do not kill the insects directly. That is why the useful insects (parasites and predators) are protected or conserved. Approaches of this kind accelerate the integration of parasites and predators with neem's compounds without leaving any toxic residues (Brown, 2006). It has documented in the literature that efficacy of neem product could be enhanced when extracted in different organic solvents (Badshah *et al.*, 2015; Aziz *et al.*, 2013).

METHODS AND MATERIALS

Mealybug culture preparation

To maintain the culture of mealybug, China rose (*Hibiscus rosa-sinensis* Linn) plants planted in pots were taken from the Horticulture Landscape Nursery, The University of Agriculture, Peshawar. When these plants grown properly they were brought to the Department of Plant Protection laboratory. Prior to mealybug inoculation, these plants were kept in the laboratory for about 3 to 4 days so that they can tolerate the pest stress after inoculation. These plants were inoculated with cotton mealybug already confirmed through taxonomic keys which were taken from Entomology section of Agriculture Research Institute (ARI), Tarnab, Peshawar.

Collection of neem materials and preparation of its extracts

Neem seeds were taken from open Market Khyber Bazaar Peshawar, while Neem seed extraction was carried out in dichloromethane and methanol as solvent in the lab of plant protection department, The University of Agriculture Peshawar. For extract preparation neem seed were crushed with the help of a grinder. After proper crushing, two samples each of 50 g were taken in two different flasks already containing dichloromethane and methanol solvents. After shaking for a while it was kept for about 72 h. After 72 h further solvent was added and were kept for about 3 to 4 h and then passed through a filter paper. In this way the filtrate which was the mixture of extract and solvent were obtained. The whole solvent was evaporated

through rotary evaporator to get a solid paste. This paste was crude extract in which known amount of distilled water was added to make stock solution. Further concentration of 1, 2 and 3% (V/V and W/V) were prepared according to the following formula.

$$C_1V_1 = C_2V_2$$

C₁, concentration of stock solution; C₂, required concentration; V₁, volume required from stock solution for making desired concentration; V₂, required volume.

Bioassay

Surface treatment bio-assay method was used to assess the toxic effect. Methodology of Badshah *et al.* (2015) and Rashid *et al.* (2011) with some modification was used.

Toxicity tests

For the contact action of neem seed extract on growing adults' cotton mealybug, the bioassay was conducted on 2-3 inch okra fruits with cotton swab. These okras were kept in 150 ml Petri dishes having a moist whatman No. 1 filter paper in the bottom to conserve the moisture content and to avoid desiccation of the Okra fruits. Then a population of 20 mealybugs (1st instar 10 on each okra) was taken per Petri dish, already produced in the laboratory, which were repeated 5 times. These mealybugs were distributed on the Okra fruits evenly through camel hair brush carefully so that no mechanical injury could produce on its body. These mealybugs were allowed to forage and settled down properly for at least 24 h. The same procedure was repeated for 2nd instar and adult cotton mealybug.

1st instars

The okra fruits having 1st instars mealybug in the Petri dishes (each set of 5 replications) was fully sprayed by methanol neem seed extract (1%, 2%, 3%) and dichloromethane neem seed extract (1%, 2%, 3%) respectively through a hand micro sprayer in such a way that all the mealybugs could receive equal dose of the toxicants. While control was sprayed with distilled water and also repeated 5 times.

2nd instars

In the same manner 2nd instars cotton mealybug on okra fruits in Petri dishes

were sprayed by all the above mentioned doses of neem seed extract in methanol, DCM and one set of 5 replications with distilled water as a control.

Adult

The same procedure which is mentioned above was repeated for adult cotton mealybugs.

Effect on natural enemies (Aenaeus bambawalei)

To know the effect of these treatments on emergence of parasitoid, 20 numbers of pupae already produced in the laboratory were taken per Petri dish containing filter paper. These Mealybugs were sprayed with different concentrations of neem seed extracts in Dichloromethane and methanol. Its emergence was checked for each concentration of both solvents. For finding percent emergence the following formula was used;

$$\% \text{ Emergence} = \frac{\text{No of emerged pupae}}{\text{Total no of pupae}} \times 100$$

Each experiment replicated five times (n=5).

Data record

Data was recorded on daily basis after spray application and numbers of dead individuals were counted, sorted out and converted to cumulative percent mortality for each Neem seed extract treatments in both solvents.

Statistical analysis

All the data regarding the mealybug mortality in each treatment was subjected to statistical analysis through analysis of variance (ANOVA) under CRD by using Statistix 8.1 version. All the means were separated by applying the Least Significant Difference (LSD) test at 5% level of probability.

RESULTS

Toxicity of neem treatments against different instars of mealybug

First instar

Table I indicated that the highest mortality on day 1st and 2nd was recorded in 3% methanol neem seed extract which is significantly high as compared to the

control (treated with distilled water) followed by 2% methanol while other treatments caused lower mortality which were not significantly ($P>0.05$) different from each other. Similarly on 3rd day again 3% methanol caused significantly high mortality followed by 2% methanol while the remaining treatments were non-significant from each other however, significantly different from control. From day 4th till day 6th, 3% high concentration of methanol caused significantly high mortality followed by the lower concentrations. Similarly, during this period all concentrations of dichloromethane (DCM) treatments caused statistically the same mortality but significantly ($P<0.05$) different from the control.

After one week exposure to all concentrations the highest mortality was recorded in 3% methanol neem seed extract followed by 2% of the same extract, while 1% of methanol extract caused statistically similar mortality as recorded in 2% and 3% dichloromethane. Among all the concentrations, the lowest mortality was recorded in 1% dichloromethane but was significantly ($P<0.05$) high from the control. After one week exposure, the mortality was recorded as 85%, 69%, 49%, and 45% by 3%, 2%, 1% methanol neem seed extract and dichloromethane neem seed extract respectively. Table 1 also showed that efficacy of each extract increased as the concentration of the respective extracts increased as evident from the mortality which was significantly high at 3% of the both extracts.

TABLE I.- TOXICITY OF DIFFERENT DOSES OF NEEM SEED EXTRACT IN METHANOL AND DICHLOROMETHANE AGAINST 1ST INSTAR OF *P. SOLENOPSIS* (% MORTALITY, MEANS \pm SE, N=5).

Chemical	Dose %	Interval (days)						
		1	2	3	4	5	6	7
Methanol	1%	10 \pm 0.0c	21 \pm 1.0c	33 \pm 2.00c	41 \pm 2.91c	43 \pm 3.39c	48 \pm 1.22c	49 \pm 1.87c
	2%	17 \pm 2.0b	31 \pm 1.0b	43 \pm 1.22b	55 \pm 1.58b	62 \pm 1.22b	65 \pm 1.58b	69 \pm 1.87b
	3%	25 \pm 1.58a	43 \pm 1.22a	58 \pm 1.22a	70 \pm 0.0a	80 \pm 1.58a	84 \pm 1.87a	85 \pm 1.58a
DCM	1%	10 \pm 1.58c	18 \pm 2.00c	25 \pm 1.58d	32 \pm 2.00d	33 \pm 2.54d	36 \pm 2.91d	39 \pm 2.44d
	2%	12 \pm 1.22c	21 \pm 2.44c	28 \pm 2.00cd	33 \pm 2.54d	39 \pm 1.87cd	40 \pm 1.58d	43 \pm 2.54cd
	3%	13 \pm 2.0bc	22 \pm 2.0c	29 \pm 2.91cd	30 \pm 2.23d	34 \pm 2.44d	40 \pm 1.58d	45 \pm 3.16cd
Control		2 \pm 1.22d	4 \pm 1.00d	5 \pm 1.58e	8 \pm 1.22e	10 \pm 1.58e	11 \pm 1.00e	14 \pm 1.87e

Means in each column followed by the same letters are not significantly different at 5 % level of probability. DCM, Dichloromethane

Percent mortality in comparison with control (Table II) showed that in all these treatments mortality increased with the passage of time exposure and highest mortality was recorded at day 3 and was then decreased gradually and lowest mortality was recorded on last day of the week.

Second instars

Table III indicated that after one day of exposure, methanol neem seed extract in 3% concentration caused highest mortality followed by 2% concentration, while remaining four treatments caused statistically similar ($P>0.05$) mortality however, significantly ($P<0.05$) different from the control. On day-2 highest mortality was recorded in 2 and 3% concentrations of methanol neem seed extract followed by 1% methanol neem seed extract while dichloromethane caused statistically the same mortality in all the three concentrations. On 3rd day again the highest mortality was recorded at 3% methanol neem seed extract followed by 2 and 1% concentrations. Dichloromethane neem seed extract again caused statistically the same mortality in all the three concentrations. From day-4 till day-7 methanol caused highest mortality followed by dichloromethane and was significantly ($P<0.05$) different from the control. After one week exposure to all concentrations of both extracts mortality of 2nd instars CMB was recorded 85%, 72%, 51% and 43% by 3%, 2%, 1% methanol neem seed extract and 3% dichloromethane neem seed extract respectively.

TABLE II.- MORTALITY (PERCENT) OF 1ST INSTARS *PHENACOCCLUS SOLENOPSIS* IN COMPARISON WITH CONTROL BY DIFFERENT CONCENTRATION OF NEEM SEED EXTRACT IN METHANOL AND DICHLOROMETHANE. (ABBOTT FORMULA)

Chemical	Dose%	Interval (days)						
		1	2	3	4	5	6	7
Methanol	1%	80.00	80.95	84.85	80.49	76.74	77.08	71.43
	2%	88.24	87.10	88.37	85.45	83.87	83.08	79.71
	3%	92.00	90.70	91.38	88.57	87.50	86.90	83.53
DCM	1%	80.00	77.78	80.00	75.00	69.70	69.44	64.10
	2%	83.33	80.95	82.14	75.76	74.36	72.50	67.44
	3%	84.62	81.82	82.76	73.33	70.59	72.50	68.89

Abbott formula: Mortality (%) = $(Mo - Mc)/Mo \times 100$

Where Mo is observed mortality (%) in treated Petri dishes and Mc is controlled mortality (%) in untreated (i.e. control) Petri dishes.

Table IV showed that mortality recorded was dose dependent and mortality increased with the increased in concentration of both extracts as highest mortality was recorded in 3% (highest concentration) concentrations.

TABLE III.- TOXICITY OF DIFFERENT DOSES OF NEEM SEED EXTRACT IN METHANOL AND DICHLOROMETHANE AGAINST 2ND INSTARS *P. SOLENOPSIS* (% MORTALITY, MEANS±SE, n=5).

Chemical	Dose %	Interval (days)						
		1	2	3	4	5	6	7
Methanol	1%	14±1.87c	28±2.00b	36±2.44c	43±2.00c	46±1.87c	47±1.22c	51±1.87c
	2%	20±2.23b	36±1.87a	47±2.00b	55±3.53b	64±3.31b	68±3.00b	72±2.54b
	3%	25±1.58a	39±1.00a	54±1.87a	65±2.73a	74±2.44a	80±2.23a	85±1.58a
DCM	1%	10±1.58c	16±1.00c	22±1.22d	26±1.87d	33±2.54d	37±2.54d	39±1.87d
	2%	11±1.00c	17±1.22c	23±1.22d	29±1.87d	34±1.00d	36±1.00d	39±1.87d
	3%	13±1.22c	19±1.87c	24±2.44d	30±1.58d	33±2.54d	38±3.00d	43±2.54d
Control		3±1.22d	5±1.58D	7±1.22e	8±1.22E	11±1.0e	13±1.2e	15±1.5e

Means in each column followed by the same letters are non-significant at 5% level, using LSD test.

TABLE IV.- MORTALITY (PERCENT) OF 2ND INSTARS *P. SOLENOPSIS* IN COMPARISON WITH CONTROL BY DIFFERENT CONCENTRATION OF NEEM SEED EXTRACT IN METHANOL AND DICHLOROMETHANE. (ABBOTT FORMULA).

Chemical	Dose%	Interval (days)						
		1	2	3	4	5	6	7
Methanol	1%	78.57	82.14	80.56	81.40	76.09	72.34	70.59
	2%	85.00	86.11	85.11	85.45	82.81	80.88	79.17
	3%	88.00	87.18	87.04	87.69	85.14	83.75	82.35
DCM	1%	70.00	68.75	68.18	69.23	66.67	64.86	61.54
	2%	72.73	70.59	69.57	72.41	67.65	63.89	61.54
	3%	76.92	73.68	70.83	73.33	66.67	65.79	65.12

Mortality of adult cotton mealy bugs

Table V showed that on day-1, the highest mortality of adult cotton mealybug was recorded at 3% and 2% methanol neem seed extract followed by the 1% of the same extract and 3% and 2% DCM neem seed extract. On day-2 and 3 the highest mortality was recorded at 3% and 2% methanol neem seed extract which were non-significant with each other and highly significant with

the control, followed by 1% of the same extract and 3% DCM neem seed extract. 1% methanol neem seed extract and 3% DCM neem seed extract are also non significant to each other but are significant to control. On day 4 the highest mortality was recorded again at 3% and 2% methanol neem seed extract followed by 1% of the same extract, 3% and 2% DCM neem seed extract. 3% and 2% methanol neem seed extracts were not significantly different to each other but were highly significant to control.

TABLE V.- TOXICITY OF DIFFERENT DOSES OF NEEM SEED EXTRACT IN METHANOL AND DICHLOROMETHANE AGAINST ADULT STAGE OF *P. SOLENOPSIS* (% MORTALITY, MEANS \pm SE, n=5).

Chemical	Dose %	Interval (days)						
		1	2	3	4	5	6	7
Methanol	1%	7 \pm 1.22b	17 \pm 2.54b	24 \pm 2.91b	32 \pm 3.39b	33 \pm 4.06b	36 \pm 3.31b	38 \pm 4.06b
	2%	12 \pm 1.22a	25 \pm 2.73a	36 \pm 1.87a	42 \pm 1.22a	51 \pm 2.44a	55 \pm 2.23a	64 \pm 4.84a
	3%	15 \pm 1.58a	24 \pm 1.87a	38 \pm 1.22a	45 \pm 3.16a	52 \pm 3.00a	61 \pm 2.91a	65 \pm 3.53a
DCM	1%	5 \pm 1.58bc	8 \pm 1.22c	10 \pm 1.58c	13 \pm 2.00de	15 \pm 3.16cd	19 \pm 2.44c	21 \pm 1.87c
	2%	3 \pm 1.22bc	8 \pm 1.22c	14 \pm 1.00c	15 \pm 1.58d	17 \pm 1.22c	20 \pm 2.23c	23 \pm 2.00c
	3%	6 \pm 1.87b	14 \pm 1.87b	20 \pm 2.2b	23 \pm 3.39c	28 \pm 2.00b	30 \pm 1.58b	33 \pm 3.00b
Control		1 \pm 1.00c	2 \pm 1.22d	4 \pm 1.87d	6 \pm 1.00e	9 \pm 1.00d	11 \pm 1.00d	11 \pm 1.00d

Means in each column followed by the same letters are non significant at 5% level, using LSD test.

Percent mortality recorded on day-5 till day-7 showed that the highest mortality was recorded at 3% and 2% methanol neem seed extract which are non significant to each other but are highly significant then control, which are followed by 1% of the same extract and 3% of DCM neem seed extract. There is no significance in percent mortality among 1% methanol neem seed extract and 3% DCM neem seed extract but were significantly different then control. After overall one week exposure the highest mortality was recorded at 3% and 2% methanol neem seed extracts followed by 1% of the same extract, 3% and 2% of DCM neem seed extract which are 65%, 64%, 38%, 33% and 23% respectively. It also showed that increasing the concentration of the respective extract, increase the percent mortality as highest mortality was recorded at 3% of both extracts. Table VIII indicated that percent mortality was high on first two days and then decreased with the time interval and the lowest percent mortality was recorded during the last day of the week (day-5).

TABLE VI.- PERCENT MORTALITY OF ADULT STAGE *P. SOLENOPSIS* IN COMPARISON WITH CONTROL BY DIFFERENT CONCENTRATION OF NEEM SEED EXTRACT IN METHANOL AND DICHLOROMETHANE.

Chemical	Dose%	Interval (days)						
		1	2	3	4	5	6	7
Methanol	1%	85.71	88.24	83.33	81.25	72.73	69.44	71.05
	2%	91.67	92.00	88.89	85.71	82.35	80.00	82.81
	3%	93.33	91.67	89.47	86.67	82.69	81.97	83.08
DCM	1%	80.00	75.00	60.00	53.85	40.00	42.11	47.62
	2%	66.67	75.00	71.43	60.00	47.06	45.00	52.17
	3%	83.33	85.71	80.00	73.91	67.86	63.33	66.67

Effect of neem extracts on percent emergence of Aenaeus bambawalei

Table VII showed that the percent emergence from the pupae of *Aenaeus bambawalei* was not affected by different doses of neem seed extracts in methanol and dichloromethane as all the data recorded was statistically non significant ($P>0.05$).

TABLE VII.- PERCENT EMERGENCE FROM THE PUPAE OF *A. BAMBAWALEI* TREATED WITH DIFFERENT DOSES OF METHANOL AND DICHLOROMETHANE NEEM SEED EXTRACT.

Chemical	Doses %	Interval (days)							
		1	2	3	4	5	6	7	8
Methanol	1%	16±2.44	30±3.16	48±2.00	64±4.00	74±2.44	76±2.44	76±2.44	76±2.44a
	2%	16±2.44	38±2.00	50±3.16	62±2.00	74±2.44	78±3.74	78±3.74	78±3.74a
	3%	16±5.09	34±5.09	46±2.44	58±3.74	70±5.47	72±4.89	72±4.89	72±4.89a
DCM	1%	18±4.89	36±2.44	46±5.09	58±3.74	64±5.09	72±3.74	72±3.74	72±3.74a
	2%	20±3.17	36±2.44	48±3.74	60±3.16	70±3.16	78±3.74	78±3.74	78±3.74a
	3%	18±3.74	36±2.44	46±2.44	60±3.16	74±2.44	80±3.16	80±3.16	80±3.16a
Control		18±5.83	38±4.89	52±3.74	68±3.74	78±3.74	80±3.16	80±3.16	80±3.16a

Effect of different neem extracts on parasitism of A bambawalei

Table VIII showed that percent parasitism of *Aenaeus bambawalei* was not affected when exposed to different treatments. Statistically the *Aenaeus*

bambawalei parasitized the same number of cotton mealybug after treated by different treatments including control, which indicated that there is no significant ($P>0.05$) effect of different neem extracts on the parasitism of the parasitoid, *Aenaeus bambawalei*

TABLE VIII.- PERCENT PARASITISM BY *AENAEIUS BAMBAWALEI* ON TREATED COTTON MEALYBUG.

Chemical	Dose %	Parasitism %
Methanol	1	14±2.44
	2	12±2.00
	3	10±0.00
DCM	1	12±2.00
	2	12±2.00
	3	10±0.00
Control		14±2.44

N.S

DISCUSSION

Neem products have toxic effect on insect pest which depends upon the product concentration and the target insect species (Schmutter, 1995; Amaugo and Emosairue, 2003) however, the main ingredient which have repellent and insecticidal action against many insect pests is azadirachtin (Schmutter, 2002). Neem oil and water extracts have also been used against insect pest under laboratory and field conditions by various researchers (Sadre *et al.*, 1983; Datta *et al.*, 2010). It is also reported that it has detrimental effects on the physiology and behavior of different species of Homoptera and Hemiptera (Nathan *et al.*, 2005a; Schmutter and Singh, 2002).

The present study was conducted to evaluate the toxic effect of different concentrations of neem seed extract against four different life stages (1st, 2nd instars and adults) of cotton mealybug, *Phenacoccus solenopsis* Tinsley. Extraction was carried out in two different solvents, methanol and dichloromethane (DCM) so that to evaluate and enhance its toxicity (Aziz *et al.*, 2013; Badshah *et al.*, 2015).

Our results showed that higher concentration of both neem extracts caused significantly high mortality in 1st instar cotton mealybug. These results are at par with the findings of Rashid *et al.* (2012) however, he used different solvents but reported highest mortality in higher concentrations of neem extracts. These

extracts of neem seeds in methanol and dichloromethane have also showed good toxic effects against 2nd instar cotton mealybug. Similarly, like 1st instar again significantly high mortality occurred in highest concentration (3%) in both neem extracts followed by lower concentrations. Results also showed that in both extracts of all concentration mortality gradually increased up to day 4 and then gradually decreased because lowest mortality was recorded at last day of the week. Similar results also reported by Khan *et al.* (2002), who found that there was gradual decrease in the efficacy of neem derivatives against the target insect pest as their efficacy was very less after 168 as compared to 24 after spray. The reduction in the efficacy is due to the deterrent and antifeedants effect of neem derivatives on sucking insect pests therefore, they are forced to leave the target place (Khattak *et al.*, 2006).

Our results for neem seed extract in methanol and dichloromethane against adult cotton mealybug showed that the highest mortality was recorded in 3% and 2% methanol neem seed extract followed by other lower concentrations. Islam (1983) found that there is significant deterrence effect on feeding of brown plant hopper and rice hispa in treatment of hexane extract of neem further he also found that ether and hexane extract of neem seed reduce the egg deposition of brown plant hopper and feeding by rice hispa in rice young seedlings. It also showed a gradual decrease in percent mortality of adult cotton mealybug with time and the highest mortality was recorded after one day of spray application and lowest on the last day of the week. The results also indicate that increase in mortality occur by increasing the respective concentrations.

Overall, our results showed that in both neem seed methanol extract and dichloromethane against all the four different stages of cotton mealybug, the methanol neem seed extract was found more toxic having significantly high cumulative percent mortality as compared to dichloromethane. Moreover, among all the concentrations the highest mortality was found in 3% dose means mortality was observed as dose dependent. These results are in accordance with the results of Badshah *et al.* (2015) who also reported a dose dependent mortality in different neem extracts. Our results also showed that with the passage of time both the extracts showed a gradual reduction in the percent mortality. Our results are in consistence with Aziz *et al.* (2013) who used ethanol and n-hexane neem seed extracts. However, here our results are in contrast with Rashid *et al.* (2012) who reported that neem toxicity remained unaffected up to three months. We suggest that the solvents we used could reduce the toxicity time of the neem extracts.

Compatibility with natural enemies

In order to check the compatibility of neem seed methanol and dichloromethane extracts with natural enemy, *Aenasius bambawalei* Hayat, a parasitoid of cotton mealybug. Two experiments were carried out. In the 1st experiment the parasitism preference of the parasitoid towards the cotton mealybug, already treated by these extracts were evaluated while in another set of experiment percent emergence was checked from the treated pupae of *Aenasius bambawalei* in all the concentrations of both neem seed extract and control. Results showed no negative impact on percent parasitism by the parasitoid or percent emergence of the parasitoid among treated and control. So overall, results of both the experiments on natural enemies (*Aenasius bambawalei*) show that both neem seed extracts are safe for natural enemies. Similar results also reported by Aziz *et al.* (2013) who checked the neem seed kernel extracts against mummified aphids, lady bird beetle, green lacewing and syrphid fly. Similarly very little impact recorded on the emergence, survival and development of aphid parasitoid when treated with neem seed extract, the emergence of parasitoid was similar in treated and untreated citrus brown aphids (Tang *et al.*, 2002). Similarly Vogt (1993) reported no significant influence of neem in the field trial of neem-Azal-F on green lacewing, further he added that the products of neem are effective when it is ingested by insects which feed on tissue of plant, while those which feed on other insects were not much affected, which may easily confirm the insensitivity of neem products.

CONCLUSION AND RECOMMENDATIONS

From the current studies it was concluded that overall mortality rate was doze and time dependent and all the treatments control the pest up to some extent but 3 % methanol Neem seed extract go faraway than the dichloromethane extracts so this concentration may be applied for the control of the mealybug. Besides, further study is needed to make the Neem extracts through more sophisticated/scientific methods in different other solvents to enhance its efficacy and then check its toxicity against cotton mealybug.

REFERENCES

- ABBAS G., ARIF, M.J, SAEED, S. AND KARAR, H., 2009. A new invasive species of genus *Phenacoccus Cockerell* attacking cotton in Pakistan. *Int. J. Agric. Biol.*, **11**: 54-58
- AHMAD F., AKRAM, W. SAJJAD, A. AND IMRAN, A.U., 2011. Management practices against cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). *Int. J. Agric. Biol.*, **13**: 547-552

- AKINTOLA, A. J. AND ANDE, A. T., 2009. Life history and behaviour of *Rastrococcus invadens* Williams on *Ficus thonningii* in Nigeria. *Aust. J. Crop Sci.*, **3**: 1-5.
- AMAAUGO, G.O. AND EMOSAIRUE, S.O., 2003. The efficacy of some indigenous medicinal plants extracts for the control of upland stem borers in Nigeria. *Trop. Subtrop. Agroecosyst.*, **2**: 121-127.
- ARIF, M.I., RAFIQ, M. AND GHAFAR, A., 2009. Host plants of cotton mealybug (*Phenacoccus solenopsis*): a new menace to cotton agroecosystem of Punjab, Pakistan. *Int. J. Agric. Biol.*, **11**: 163-167.
- AZIZ, M., AHMAD, M., NASIR, M. AND NAEEM, M., 2013. Efficacy of different neem (*Azadirachta indica*) products in comparison with imidacloprid against english grain aphid (*Sitobion avenae*) on wheat. *Int. J. Agric. Biol.*, **15**: 279-284.
- BADSHAH, H., ULLAH, F., FARID, A. CALATAYUD, P.A. AND CRICKMORE, N., 2015. Toxicity of neem seed *Azadirachta indica* Juss (Meliaceae) different solvents extracts against cotton mealybug *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) under Laboratory conditions. *J. Ent. Zool. Stud.*, **3**: 45-49.
- BROWN, A.E., 2005. Mode of action of insecticides and related pest control chemicals for production agriculture, ornamentals, and turf. *Pestic. Educ. Assess. Program.*, **301**: 405-3913.
- DATTA, S., GHOSH, A. SARKAR, S., DEKA, P., CHOUDHURI, T. AND PAL, P., 2010. Herbal mosquito repellents. *Int. J. Paharm. Sci. Biol.*, **1**: 195-202.
- GULLAN, P.J. AND KOSZTARAB, M., 1997. Adaptations in scale insects. *Annu. Rev. Ent.*, **42**: 23-50.
- HODGSON, C., GHULAM, A., MUHAMMAD, J.A., SHAFQAT, S. AND HAIDER, K., 2008. *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), an invasive mealybug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. *Zootaxa.*, **1913**: 1-35.
- ISLAM, B.N., 1983. *Pesticidal action of neem and certain indigenous plants and weeds of Bangladesh. Natural pesticides from the neem tree and other tropical plants.* pp. 263-290.
- JAIN HUA, M.O., 2003. *Longtailed mealybug* Monog. pp: 3
- JOSHI, M.D., BUTANI, P.G. PATEL, V.N. AND JEYAKUMAR, P., 2010. Cotton mealy bug, *Phenacoccus solenopsis* Tinsley—a review. *Agric. Rev.*, **31**: 113-119.
- KHAN, L., KHATTAK, M.K., AWAN, M.N. AND HUSSAIN, A.S., 2002. Comparative efficacy of neem (*Azadirachta indica*. A. Juss) extracts and certain insecticidal combinations against thrips and boll worms on cotton. *Gomal Uni. J. Res.*, **19**: 251-258.
- KHATTAK, M.K., MAMOON-UR-RASHID, S.A.S. AND ISLAM, H.T., 2006. Comparative effect of neem (*Azadirachta indica* A. Juss) oil, neem seed water extract and Baythroid TM against whitefly, jassids and thrips on cotton. *Pak. Entomol.*, **28**: 31-37.
- RASHID, M. M., ABDULLAH, M. K. AND HUSSAIN, S., 2011. Toxic and residual activities of selected insecticides and neem oil against cotton mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: eudococcidae) under laboratory and field conditions. *Mortality*, **33**: 151-155.
- MCKENZIE, JOSHI, M.D., BUTANI, P.G., PATEL, V.N. AND JEYAKUMAR, P., 2010. Cotton mealy bug, *Phenacoccus solenopsis*. *Agric. Rev.*, **31**: 113-119.
- MUTHUKRISHNAN, N., MANOHARAN, T. THEVAN, P.S. AND ANBU, S., 2005. Evaluation of Buprofezin for the management of grape mealy bug, *Maconellicoccus hirsutus* (Green). *J. entomol. Res.*, **29**: 339-344.

- NATHAN, S.S., KALAIVANI, K. MURUGAN K. AND CHUNG, P.G., 2005. The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenee) the rice leaf folder. *Pestic. Biochem. Physiol.*, **81**: 113-122.
- OSBORNE, L.S., PETITT, F.L., LANDA, Z. AND HOELMER K.A., 1994. *Biological control of pests attacking crops grown in protected culture: the Florida experience*.
- PATIL, S.V., PATIL, C.D., SALUNKHE, R.B., MAHESHWARI, V.L. AND SALUNKE, B.K., 2011. Studies on life cycle of mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), on different hosts at different constant temperatures. *Crop Prot.*, **30**:1553-1556.
- PRISHANTHINI, M. AND LAXMI, V.M., 2009. The *Phenococcus solenopsis*. Department of Zoology, Eastern University, Sri Lanka. Available at <http://www.dailynews.lk/2009/07/01/fea30.asp>
- SADRE, N.L., DESHPANDE, V.Y., MENDULKAR, K.N. AND NANDAL, D.H., 1983. Male *Azadirachta indica* in different species. Proc. 2nd international neem conference. Rauischholzhausen. P. 482.
- SAINI, R.K., RAM, P. SHARMA, S.S. AND ROHILLA, H.R., 2009. Mealybug, *Phenacoccus solenopsis* Tinsley and its survival in cotton ecosystem in Haryana. In: *Proc National Symposium on Bt-Cotton: Opportunities and Prospectus*. Central Institute of Cotton Research, Nagpur, November pp. 17-19.
- SCHMUTTER, H. AND SINGH, R.P., 2002. List of insect pest susceptible to neem products. In: *The neem tree Azadirachta indica A. juss. and other meliaceous plants*. VCH publications, Germany. pp. 411-156.
- SCHMUTTER, H., 1995. *The neem tree source of unique natural products for IPM, Medicine, Industry and other purposes*. VCH publications New York, USA.
- SCHMUTTER, H., 2002. *The neem tree Azadirachta indica A. juss and other meliaceous plant sources of unique natural products for integrated pest management, medicines and other purposes*. 2nd Ed. Neem Foundation Mumbai, India. pp. 893.
- SCHMUTTERER, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annu. Rev. Ent.*, **35**: 271-297.
- SHAHID., 1999. *Principles of insect pest management*. Pub. No. 19(233)/ Mono/HEC/96., pp. 183.
- SURESH, S., JOTHIMANI, R., SIVASUBRAMANIAN, P., KARUPPUCHAMY, P., SAMIYAPPAN R. AND JONATHA. E.I., 2010. Invasive mealybugs of Tamil Nadu and their management. *Karnataka J. agric. Sci.*, **23**: 6-9.
- TANG, Y.Q., WEATHERSBEE, A.A. AND MAYER, R.T., 2002. Effect of neem seed extract on the brown citrus aphid (Homoptera: Aphididae) and its parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae). *Environ. Ent.*, **31**: 172-176.
- VOGT, H., 1993. Einsatz von Niempräparaten gegen Adoxophyes orana F.V.R. und Untersuchungen zu Nebenwirkungen. In: *6th Intern. Erfahrungsaust. über Forschungserg. zum Ökologischen Obstbau*, LVWO, Weinsberg., pp: 51-55.
- WANG, Y.P., WU, S.A. AND ZHANG, R.Z., 2009. Pest risk analysis of a new invasive pest *Phenacoccus solenopsis*l, to China (in Chinese; Summary in English). *Chinese Bull.Ent.*, **46**: 101-106.
- WILLIAMS, D. J. AND GRANARA de WILLINK M.C., 1992. *Mealybugs of Central and South America*, CAB Int. p. 635.

**NEW DISTRIBUTION RECORD OF HIMALAYAN WOLF SNAKE
(*LYCODON MACKINNONI* WALL, 1906) IN AZAD KASHMIR
(PAKISTAN)**

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Abstract.- The present study was conducted to document and make checklist of herpetofauna of district Bagh (Azad Kashmir, Pakistan). The survey was conducted from May 2016 to November 2017. We collected 3 specimens of Himalayan endemic wolf-snake species from three different sites (Pannyali, Chaman Kot, Danna) of district Bagh by using the visual encounter method. The presence of (*Lycodon mackinnoni* Wall, 1906) are new distributional records, first finding and addition of new species in checklist of ophidian fauna both, for the Azad Kashmir area and Pakistan.

Key words: Western Himalayan, endemic species, conservation, Azad Kashmir, Himalayas, anthropogenic pressure.

INTRODUCTION

Serpentine fauna are diverse group of vertebrates, worldwide in distribution perform a variety of functions in earth ecosystem and provide numerous services to humanity. The services provided by serpentine fauna are, nutrient cycling, bioturbation; regulating: biological control, seed dispersal; provisioning: protein sources (food source), raw materials and medicinal resources; and cultural (Fisher *et al.*, 2009).

Natural ecosystems are facing a rapid decline of biodiversity around the globe, which has critical implications on ecosystem functions and services. Successful conservation efforts to slow this decline rely on the ability to monitor species and understand their ecological role. Such efforts are often hindered by a lack of knowledge regarding arcane interactions. Snakes provide several key regulating and supporting ecosystem services, including insect suppression, nutrient cycling and predator/prey in aquatic and terrestrial ecosystems to transfer energy between the two systems (Nowak *et al.*, 2008). However, trophic interactions that occur in both natural and anthropogenically impacted systems

remain largely obscured. Exploring the diversity, diet, and anthropogenic pressure of snake distributions along elevation gradient and diet can also aid in the understanding of their foraging ecology, which will guide future management.

In territories of Azad Kashmir (Pakistan), snakes are represented by 25 species and are facing anthropogenic, climatic, and natural predator pressures. The serpentine fauna of Himalayas have been studied by various worker such (Smith, 1943; Khan and Khan, 1996; Faiz *et al.*, 2016; Faiz, 2017) and reported 14 species of snakes in territory of Azad Kashmir.

The *Lycodon* taxa of snake is the most widely distributed taxa, ranges from the Trans Caspian Sea, Iran, China, Indo-Australian archipelago and Ryukyu Islands (Gupta and Peshin, 2014). The *Lycodon* taxa have 25 species and 11 of the mare endemic to Indian subcontinent (Mukherjee and Bhupathy, 2007). In Indian range of Himalayas, the species (*Lycodon mackinnoni*) is reported at (District Doda of Indian Kashmir) (Manhas *et al.*, 2015). A lot of studies on biodiversity of Tolipir landscape of Pir Punjal range of Lesser Himalays such as mammalian diversity (Faiz *et al.*, 2016), but the present study was designed with objective to make check list of distribution range of serpentine fauna no previous study about the distributional range of serpentine is present.

MATERIALS AND METHODS

Study area

The state of Azad Kashmir is famous for its scenic beauty and fascinating landscape, having permanent snowfields with alpine and sub-alpine vegetation, coniferous forests, and scrub forests, temperate plantations, dotted with rain-fed agriculture and limited riverian forests with elevation range 500 m asl to 6500 m asl (Fig.1).

We conducted a study to document snake mortality in rural areas of the Bagh district (Azad Kashmir, Pakistan) by visual encounter methods (Heyer *et al.*, 1994). On 20 June 2017 at 12:10 h, we found three sample of an *Lycodon mackinnoni* specimen of at sites (Pannyali, Chaman Kot, Danna). We collected and photographed the specimen. The sites were marked with GPS (Garmin arco 4.2). The soil sample was taken for further analysis. The collected specimen were preserved in 70% alcohol and identified by taxonomic keys by following (Smith, 1943).



(A)



(B)



(C)

Fig. 1. Sample collected at Danna (A), Chaman Kot (B), and Pannyali (C)

RESULTS AND DISCUSSION

The two sample of species were found on road killed accident at site (Pannyali, Danna) while third sample was found killed in the vicinity of house Chaman Kot.

Lycodon mackinnoni has not been reported in ophidian fauna of western Himalayas in Azad Jammu and Kashmir as well as in the check list of ophidian fauna of Pakistan (Khan and Khan, 1996).

The morphometric measurements (Table I) of total body length (437, 432, 426 mm) verify the length described in previous literature (Manhas *et al.*, 2015). The snout length of collected samples is nearly same (354, 353 and 351 mm) are also verified by previous literature (Manhas *et al.*, 2015). Other morphometric measurements such as, tail length, head width and eye diameter, distance between eyes are also verified by literature as reported by Manhas *et al.* (2015).

TABLE I.- MORPHOMETRIC DATA (ALL MEASUREMENTS IN mm).

Characteristics	Pannyali	Danna	Chaman Kot
Head length	12	11	10
Eye diameter	0.95	0.85	0.89
Distance b/w eyes	3.9	3.7	3.8
Tail length	80	79	78
Head width	5.2	5.1	5.0
Total body length	437	432	426
Snout vent length	354	353	351

The distributional range of the species Himalayan wolf snake, was previously recorded from Indian Kashmir (Manhas *et al.*, 2015), and present finding is a newly recorded range of *L. mackinnoni* at Tolipir landscape of Pir Punjal in Himalayas in Kashmir (Pakistan). The distributional range of species (Himalayan wolf snake) was also reported at Himalayas Mussoorie hills, Alomar and Muktesar (Smith, 1943).

The soil type was clay loam and major type of vegetation among shrubs (*Berberis lyceum*, *Clematis buchananiana*, *Heracleum*, *Rubus fruticosus*, *Viburnum grandiflorum*) and among tree (*Acer pentapomicum*, *Ficus palmate*, *Pyrusmalu*) and among grasses (*Brachiaria* spp, *Stipasibirica*, *Poanepalensis*)

among ferns (*Dryopteris juxtaoposita*, *Equisetum arvense* Linn) and the vegetation type is similar as described by (Faiz *et al.*, 2014).

TABLE II.- PHOLIDOSIC COUNT OF COLLECTED SPECIMEN.

Scalation	Number of samples				
	1	2	3	4	5
Supra labial	8	8	8	8	8
Infralabial	7	7	7	7	7
Supraocular	2	2	2	2	2
Parietal	2	2	2	2	2
Prefrontal	2	2	2	2	2
Preocular	1	1	1	1	1
Post ocular	1	1	1	1	1
Loreal	1	1	1	1	1
Intranasal	2	2	2	2	2
Temporal	5(2+3)	5(2+3)	5(2+3)	5(2+3)	5(2+3)
Dorsal body scale	17–15	17–15	17–15	17–15	17–15
Ventrals	193(163– 187)	193(163– 187)	193(163– 187)	193(163– 187)	193(163– 187)
Subcaudals	53 pairs	53 pairs	53 pairs	53 pairs	53 pairs

The area is under huge anthropogenic pressure due to exploitation of natural resources such as overgrazing of castles, timber wood, fuel wood, as worked by (Faiz *et al.*, 2015). The snake mortality rate is high (Faiz, 2017) due to fear of snake bite, and population dynamic is declining readily and need urgent need for conservation for welfare of snake.

REFERENCES

- FAIZ, H. A., 2017. Small mammals, human caused snake mortality and snake conservation in the landscape of Tolipir Azad Kashmir Pakistan. *Biology of snake.*, **36**: 13–22.
- FAIZ, H. A. AND ABASS, F., 2016. Mammalian diversity of Tolipir National Park, Azad Jammu and Kashmir, Pakistan. *Pakistan J. Zool.*, **48**: 1209–1212.
- FAIZ, H. A., FAKHAR-I-ABBAS AND ZAHRA, L., 2015. Anthropogenic influences on the tolipir landscape, lesser Himalayas, Pakistan. *J. Bioresour. Manage.*, **2**: 20–27.
- FAIZ, H. A., FAKHAR-I-ABBAS AND ZAHRA, L., 2016. Herpetofaunal diversity of tolipir national park, Azad Jammu and Kashmir, Pakistan. *Proc. Pakistan Congr. Zool.* **36**: 13–22.
- FAIZ, H. A., GHUFARN, M. A., MIAN, A. AND AKHTAR T., 2014. Floral diversity of Tolipir National Park (TNP), Azad Jammu and Kashmir, Pakistan. *Biologia (Pakistan)*, **60**: 43–55.
- FISHER, B., TURNER, K. R. AND MORLING, P., 2009. Defining and classifying ecosystem services for decision making. *Ecol. Econ.*, **68**: 643–653.

- GUPTA, K. P. AND PESHIN, S. S., 2014. Snake bite in India: Current scenario of an old problem. *J. Clin. Toxicol.* 4:1000182.dot 10.4172/2161-0495.1000182
- HEYER, W. R., DONNELLY, M. A., MCDIARMID, R. W., HAYEK, L.C. AND FOSTER, M. S., 1994. *Measuring and monitoring biological diversity: Standard methods for amphibians*. Smithsonian Institution Press, Washington and DC: p. 364.
- KHAN, A. Q. AND KHAN, M. S., 1996. Snakes of State of Azad Jammu and Kashmir. *Proc. Pakistan Congr. Zool.*, **16**: 173 – 182.
- MANHAS, A., WANGANEAO, R. N. AND WANGANEAO, A., 2015. First record of Himalayan Wolf Snake (*Lycodon mackinnoni* Wall, 1906) in Doda District of Lower Himalayas, Jammu and Kashmir, India. *World Res. J. Biol. Sci.*, **1**: 2-4.
- MUKHERJEE, D. AND BHUPATHY, S., 2007. A new species of wolf snake Serpentes: Colubridae: *Lycodon*) from Anaikatti Hills, Western Ghats, Tamil Nadu, India. *Russian J. Herpetol.*, **14**: 21–26.
- NOWAK, E. M., THEIMER, T. C. AND SCHUETT, G. W., 2008. Functional and numerical responses of predators: Where do vipers fit in the traditional paradigms? *Biol. Rev.* **83**: 601–620.
- SMITH, M. A., 1943. *The fauna of British India, Ceylon and Burma. Reptilia and amphibian serpentes*. Taylor and Francis, London. reprinted, p. 583.

WINTERING WATERFOWL DIVERSITY AT RAWAL LAKE, ISLAMABAD

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Abstract.- During current study, 40 bird species from 9 families were recorded through five months survey of lake. These families included *Anatidae* (64 %), *Phalacrocoracidae* (11%), *Ardeidae* (10.5%), *Laridae* (8%), *Charadriidae* (2.16 %), *Scolopacidae* (1.59 %), *Rallidae* (1.57 %), *Podicipedidae* (0.97%) and *Recurvirostridae* (0.24 %). According to Shannon Diversity Index, Lake hold a moderate bird diversity i.e. $H' = 2.6588$. Maximum number of birds was observed in February 2017 i.e. 1,682 individuals, while minimum number was recorded in December 2016 (91 birds). In migratory waterfowls, Northern Shoveller was most abundant species ($n = 736$, $R.A = 0.1992$, $H' = -0.3213$) and Common Pochard ($n = 2$, $R.A = 0.0005$, $H' = -0.0038$) with least abundant species recorded. In local migrant, Black headed gull was recorded with highest numbers ($n = 298$, $R.A = 0.0806$, $H' = -0.2029$) while, two birds species viz. crested great grebe and common red shank ($n = 1$, $R.A = 0.0002$, $H' = -0.0017$) were lowest in numbers.

Key words: Rawal Lake, Migratory birds, Wetlands, Waterfowl, Shannon-Wiener diversity index

INTRODUCTION

Wetlands are globally significant ecosystems (Khan and Arshad, 2014) as they provide feeding, nesting and breeding grounds to wetland's associated biodiversity. Avifauna is a key component of wetlands related biodiversity. Wetlands provide necessary habitat for nesting, foraging and social interactions thus increasing the waterfowl diversity and abundance. A huge diversity of residents as well as migratory birds is found in the wetlands of Pakistan (Grewal, 1993). Every year, during winters, large number of birds from Central Asia and Europe migrate towards Pakistan. Being at the junction of Asia's main Palearctic migration course, Pakistan supports a significant diversity of migratory flocks. Birds from their Northern breeding grounds fly to Pakistan. Migratory birds fly through Indus Flyway to reach Pakistan i.e. over the Karakorum, Suleiman and Hindu Kush Ranges along River Indus (Ali, 2006; Rais, 2009). Important migratory birds include ducks, geese, cranes, flamingos, swans, waders and falcons etc (Ali, 2005).

There are 225 wetlands of high global significance in Pakistan. They cover approximately 9.7% of land surface area and span over 780,000 ha. (Ali and Akhtar, 2006). There is a huge data gap about the biological diversity at important wetland sites in Pakistan (Li and Mundkar, 2004). Over exploitation, habitat degradation and aquatic pollution are the key factors that threaten this rich biological capital (Sheikh and Kashif, 2006). Constructions of residential areas around aquatic habitats, recreational activities, fishing practices and water contamination altogether have greatly modified the conditions of the wetlands (Hussain *et al.*, 2002; Riaz, 2004).

Situated in the Southeast of Islamabad, Rawal Lake is an important wetland of Margallah Hills National Park (Bilal *et al.*, 2011). Keeping the above facts in view, present study was planned to estimate diversity of wintering waterfowls at Rawal Lake as assessment of avian fauna may suggest measures for habitat protection and conservation.

MATERIALS AND METHODS

Study area

Rawal Lake (33° 42' 9.5868", 73° 7' 33.9816" E) is an artificial reservoir with 106 square miles of catchment area. Rawal Lake is formed by damming the Kurrang River. During an average rainfall year (1000 mm), lake has a storage capacity of 84,000 acre feet. Four major streams along with numerous small streams originating from Himalayan Foothills Region in Islamabad are source of water for the lake. Climatically area falls in humid subtropical zone with temperature ranges from 1–15°C in winter and 20–40°C in summer (Farooq and Ghalib, 1986). Total area of the lake is 1902 hectares with a buffer zone of 2 km (Hussain *et al.*, 2002).

Survey methodology

Bird monitoring surveys were carried out from November 2016 to March 2017 at three selected sites around the lake. Birds were observed from hidden observation positions at 10 to 20 meters from the water edge. Birds were recorded at or flying condition over the water body during the period of high bird activity (6.00 am to 10.00 am in morning and 4.30 pm to 7.00 pm in late afternoon). Birds were observed using spotting scopes (Nikon w/ 15-45 X) and binoculars (Olympus 8-16 X 40, DPS I). GPS (Magellan SporTrack) points of the survey sites were

recorded and periodic boat surveys were also carried out to gain a better picture of bird diversity. "Water Birds of Asia" field guide was used for bird identification. Bird data was documented instantly after viewing. The data was analyzed using Microsoft Excel 2010 to determine species richness and relative abundance. Shannon-Wiener diversity index (H) was applied to study the diversity.

$$H = - [\sum P_i \ln P_i]$$

RESULTS AND DISCUSSION

During five months survey of lake, 40 bird species with a total of 3,694 individuals (Table I) of 4 orders and 9 Families were observed. Maximum species abundance was observed in the month of February 2017 i.e. 1,682 individuals, while minimum number was recorded in December 2016 (91 birds). Ali and Akhtar (2005) reported 942 individuals of 8 duck species at Rawal Lake. In another study Zafar-Uddin *et al.*, (1983) counted as many as 2,312 individuals of 4 duck species at Rawal Lake. While Amin *et al.*, (1984) recorded 2,038 individuals of 9 duck species at this lake.

The greater abundance in February and March 2017 is due to winter visiting waterfowl. Waterfowl abundance tends to increase in winters due to migratory flocks visiting Pakistan (Shahid and Nisa, 2009). As weather warms up in plainer parts of Pakistan followed by Himalayan foot hills region, migratory flock density tends to decrease due to back migration of waterfowls.

TABLE I.- TAXONOMIC POSITION, MONTH-WISE COUNT, RELATIVE ABUNDANCE, DIVERSITY AND IUCN STATUS OF WINTERING BIRDS AT THREE FIXED SITES OF RAWAL LAKE DURING NOVEMBER 2016 TO MARCH 2017.

Sr. No.	Major Bird Taxa	Common Name	Nov.	Dec.	Jan.	Feb.	Mar.	Total	Max.	R.A*	H**	GCS***
Order: Anseriformes												
Family: Anatidae												
1	<i>Anas crecca</i>	Common Teal	0	3	38	208	62	321	208	0.087	-0.2121	LC
2	<i>Aythya ferina</i>	Common pochard	0	8	40	37	75	160	75	0.043	-0.1359	VU
3	<i>Anas platyrhynchos</i>	Mallard	0	0	150	473	12	635	473	0.172	-0.3026	LC
4	<i>Anas strepera</i>	Gadwall	0	0	50	75	48	173	75	0.047	-0.1432	LC
5	<i>Anas clypeata</i>	Northern Shoveller	0	0	56	280	400	736	400	0.199	-0.3213	LC
6	<i>Anas acuta</i>	Pintails	0	0	37	59	88	184	88	0.05	-0.1493	LC
7	<i>Anas Penelope</i>	Eurasian wigeon	0	0	15	90	3	108	90	0.029	-0.1031	LC

(continued on next page)

8	<i>Anas querquedula</i>	Garganey	0	0	0	0	35	35	35	0.009	-0.0438	LC
9	<i>Aythya fuligula</i>	Tufted duck	0	0	0	0	9	9	9	0.002	-0.0144	LC
10	<i>Aythya nyroca</i>	White Eyed Pochard	0	0	0	2	0	2	2	5E-04	-0.0038	NT
Order: Ciconiiformes												
Family: Podicipedidae												
11	<i>Tachybaptus ruficollis</i>	Little Grebe	0	0	0	29	2	31	29	0.008	-0.0397	LC
12	<i>Podiceps nigricollis</i>	Black Headed Grebe	0	0	0	0	4	4	4	0.001	-0.0069	LC
13	<i>Podiceps cristatus</i>	Crested Great Grebe	0	0	0	1	0	1	1	2E-04	-0.0017	LC
Family: Ardeidae												
14	<i>Ardeola grayii</i>	Indian Pond Heron	1	1	0	0	3	5	3	0.001	-0.0091	LC
15	<i>Ardea cinerea</i>	Grey Heron	3	4	0	0	1	8	4	0.002	-0.0134	LC
16	<i>Egretta alba</i>	Great Egret	56	5	58	78	24	221	78	0.06	-0.1684	LC
17	<i>Egretta intermedia</i>	Intermediate Egret	0	0	39	22	0	61	39	0.017	-0.0677	
18	<i>Egretta garzetta</i>	Little Egret	5	0	20	36	15	76	36	0.021	-0.0796	LC
19	<i>Bubulcus ibis</i>	Cattle Egret	10	2	0	3	2	17	10	0.005	-0.0247	LC
Family: Charadriidae												
20	<i>Vanellus vanellus</i>	Northern Lapwing	2	1	0	0	2	5	2	0.001	-0.0091	NT
21	<i>Vanellus indicus</i>	Red Wattled Lapwing	0	0	3	30	15	48	30	0.013	-0.0561	LC
22	<i>Charadrius alexandrinus</i>	Kentish Plover	0	20	0	3	0	23	20	0.006	-0.0315	LC
23	<i>Charadrius hiaticula</i>	Little Ringed Plover	3	0	1	0	0	4	3	0.001	-0.0069	LC
Family: Scolopacidae												
24	<i>Actitis hypoleucos</i>	Common Sandpiper	9	10	0	0	4	23	10	0.006	-0.0315	LC
25	<i>Tringa glareola</i>	Wood Sandpiper	0	10	0	0	2	12	10	0.003	-0.0183	LC
26	<i>Tringa nebularia</i>	Green Shank	2	0	1	0	1	4	2	0.001	-0.0069	LC
27	<i>Tringa tetanus</i>	Common Red Shank	0	0	0	0	1	1	1	2E-04	-0.0017	
28	<i>Calidris alba</i>	Sanderling	1	0	2	0	4	7	4	0.002	-0.0119	LC
29	<i>Calidris temminckii</i>	Temminck's Stint	0	0	0	2	5	7	5	0.002	-0.0119	LC
30	<i>Calidris minuta</i>	Little Stint	0	2	0	0	1	3	2	8E-04	-0.0057	LC
31	<i>Gallinago gallinago</i>	Common Snipe	0	0	0	3	2	5	3	0.001	-0.0091	LC

(continued on next page)

Family:Phalacrocoracidae												
32	<i>Phalacrocorax carbo</i>	Large Carmorent	0	3	0	189	0	192	189	0.052	-0.1535	LC
33	<i>Phalacrocorax sulcirostris</i>	Little Black Carmorent	3	0	150	50	0	203	150	0.055	-0.1593	LC
Family: Laridae												
34	<i>Lrus ridibundus</i>	Black Headed Gull	250	20	20	8	0	298	250	0.081	-0.2029	LC
35	<i>Sterna aurantia</i>	Indian River Tern	0	0	1	4	0	5	4	0.001	-0.0091	NT
Order: Charadriiformes												
Family: Recurvirostridae												
36	<i>Himantopus himantopus</i>	Black Winged Stilt	0	0	2	0	7	9	7	0.002	-0.0144	LC
			0	0	1	0	2	3	2	8E-04	-0.0057	LC
Order: Gruiformes												
Family: Rallidae												
37	<i>Amauornis akool</i>	Brown Crake	0	0	1	0	2	3	2	8E-04	-0.0057	LC
38	<i>Gallinula chloropus</i>	Indian Moorhens	1	0	4	0	2	7	4	0.002	-0.0119	LC
39	<i>Amauornis phoenicurus</i>	White Breasted Waterhen	2	0	0	3	0	5	3	0.001	-0.0091	LC
40	<i>Fulica atra</i>	Common Coot	0	0	26	0	17	43	26	0.012	-0.0516	LC
	Total Average		350	91	724	1682	847	3694	2386	0.998025	-2.659-0.066	
R.A* Relative Abundance H*** Shanon Weiner Diversity Index GCS*** Global Conservation Status												

Shannon Diversity Index (H') for each bird species was calculated (Table I). Rawal lake has moderate diversity of birds i.e. $H' = 2.6588$. Thirteen migratory bird species and 27 local migrants were observed at Rawal Lake. In migratory waterfowls, Northern Shoveller was most abundant species ($n = 736$, R.A= 0.1992, $H' = -0.3213$) and Common Pochard ($n = 2$, R.A= 0.0005, $H' = -0.0038$) was least abundant species recorded. In local migrants, black headed gull was recorded with highest numbers ($n = 298$, R.A= 0.0806, $H' = -0.2029$) while, two birds species viz. crested great grebe and common red shank ($n = 1$, R.A= 0.0002, $H' = -0.0017$) were lowest in numbers. There is a record of 15 migratory and 6 resident species at Rawal Lake. Most important species among these were long tailed duck and red-crested pochard (Hussain *et al.*, 2002). Since, there have been no records of long tailed duck within the past 50 years (Roberts, 1991). Both these bird species were not recorded during the present study.

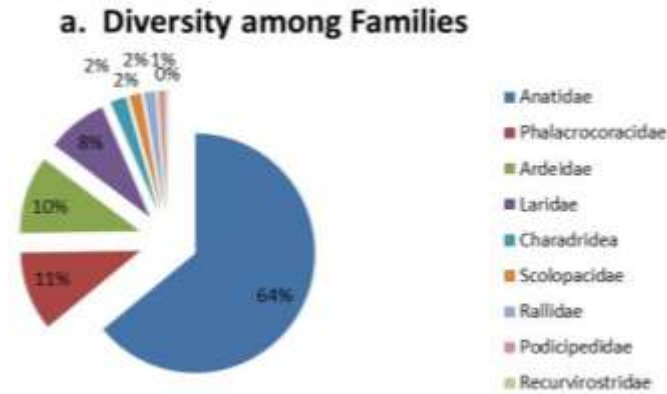


Fig. 1. Percentage of nine different Families of waterfowls observed at Rawal Lake.

In the current study, among 9 families recorded, Anatidae with ten duck species was most abundant with 2363 individuals (64 %) as shown in Figure 1. Second more abundant family with 395 individuals (11%) was Phalacrocoracidae with only two bird species. The third one was Ardeidae with 388 individuals (10.5 %) and six bird species. The fourth one was family Laridae with 303 individuals (8%) and two bird species. Family Charadriidae with 80 individuals (2.16%) and three bird species was at fifth most abundant. Least abundant families included the 62 individuals (1.59 %) of family Scolopacidae, 58 individuals (1.57 %) of family Rallidae, Podicipedidae with 36 individuals (0.97 %) and Recurvirostridae with 9 individuals (0.24 %). Bilal *et al.* (2011) reported 585 individuals of seven species of migratory ducks (Anseriformes, Anatidae) at Rawal Lake.

Threats to avifauna at Rawal Lake

Due to a broad spectrum of anthropogenic activities, wetlands are under tremendous pressure and are being degraded due to over exploitation, pollution etc. Many aquatic habitats are also a victim of human ignorance and mismanagement. Excessive fishing and construction disturbances have degenerated natural environment of Rawal Lake. Bird hunting around the Rawal Lake is a bigger concern. Lake is a recreational area. The terraced garden and the lake are used for picnics and fishing activities. It is necessary to protect the niche of migratory birds. Lake pollution also needs to be monitored along with

construction activities near the buffer zone to protect the visiting avifauna.

CONCLUSION

Rawal Lake being important habitat for waterfowl tends to get less inhabitable due to high rate of anthropogenic activities. Many sites along the lake, previously regarded as good habitats are no longer hosting bird species. There is a dire need to address the issues of bird hunting and construction in Lake buffer zone to safeguard the avian diversity. Continue bird monitoring is also required to properly document all the species at the lake and for better management of the lake fauna.

ACKNOWLEDGEMENTS

The research work was financially supported by Zoological Survey of Pakistan, Ministry of Climate Change Islamabad. I am especially indebted Director, Zoological Survey of Pakistan for providing me the opportunity to conduct field studies. Technical and logistic supportive staff of the department is highly acknowledged for their support during field studies.

Author's Contribution:

Field surveys were jointly carried out in lead of Mehrban Ali Brohi and assisted by Zuberia Anwar and Khurram Saeed.

REFERENCES

- ALI, Z. AND AKHTAR, M., 2006. *Decreases in size of lakes and numbers of birds in selected wetlands in Pakistan -Waterbirds around the world.* 294-295. The Stationery Office, Edinburgh, UK.
- ALI, Z., 2006. Avian species analysis at three major wetlands complex Pakistan. *Biologia*, **2**: 203-213.
- ALI, Z., 2005. *Ecology, distribution and conservation of migratory birds at Uchalli wetlands complex, Punjab, Pakistan.* Ph.D thesis, University of the Punjab Pakistan.
- ALI, Z. AND AKHTAR, M., 2005. Bird surveys at wetlands in Punjab, Pakistan, with special reference to the present status of white-headed duck *Oxyura leucocephala*. *Forktail*, **21**: 43-50.
- AMIN, A., ANWAR, M., AHMAD, M. AND AKBER, G., 1984. *Study on status of habitat and distribution of wildlife in Islamabad District (Margalla Hills, Bannigala and surrounding area).* Annual progress report 1983-84. PARC. Islamabad.
- BHINDER, M. A., IQBAL, M., SHAHBAZ, M., ZAHOOR, M. Y. AND SHEHZAD, W., 2015. Avian biodiversity of bajwat wetland, district Sialkot. *Pak. J. Anim. Pl. Sci*, **25**: 416-422.
- BILAL, S., RAIS, M., ANWAR, M., MEHMOOD, T., HUSSAIN, I. AND NADEEM, M. S.,

2011. Trend in the diversity of migratory ducks at Rawal Lake, Islamabad. *Researchgate*. **20**: 65-69
- GREWAL, B., 1993. *Birds of India Bangladesh, Nepal, Pakistan and Sri Lanka*. Guide Book Company limited, Hong Kong.
- HUSSAIN, S. D., GILANI, T., KHAN, R. A., ANWER, M. AND BOKHARI, R., 2002. Population of migratory and resident species at Rawal Lake, Islamabad. *Indus J. Pl. Sci.*, **1**: 283-286.
- KHAN, A. A. AND ARSHAD, S., 2014. Wetlands of Pakistan: distribution, degradation and management. *Pakistan Geogr. Rev.* **69**: 28-45.
- LI, Z. W. D. AND MUNDKUR, T., 2004. *Numbers and distribution of waterbirds and wetlands in the Asia-Pacific region*. Results of the Asian Water bird Census: 1997-2001. Wetlands International, Kuala Lumpur, Malaysia.
- RAIS, M., 2009. *Migratory birds. Letter to editor. The News Pakistan*. 11.05.2009.
- RIAZ, G., 2004. *Rawal Lake – management of a wetland ecosystem under threat. Internship report*. Department of Range Management and Forestry. University of Arid Agriculture, Rawalpindi.
- ROBERTS, T. J., 1991. *The birds of Pakistan*. Vol. 1, Oxford University Press, Karachi, Pakistan.
- SHAHID, M. AND ZAIB-UN-NISA, 2009. Diversity of avifauna of Trimmu Barrage, District Jhang, Punjab, Pakistan. *Pakistan J. Zool.* **41**: 43-49.
- SHEIKH, K. M. AND KASHIF, N., 2006. *Strategic role of Pakistan wetland resources: prospects for an effective migratory waterbird conservation network*. - *Waterbirds around the world* (eds. G.C. Boere, C.A. Galbraith and D.A. Stroud). The Stationery Office, Edinburgh, UK. 292-293.
- SONOBE, K. AND USUI, S., 1993. *A field guide to waterbirds of Asia*. Wild Bird Society of Japan, Tokyo.
- ZAFAR-UDDIN, AMIN, A., AKBER, G. AND ANWAR, M., 1983. *Study on status of habitat and distribution of wildlife in Islamabad. District (Margalla Hills, Bannigala and surrounding area)*. Annual progress report. 1982-83. PARC, Islamabad.

EFFICACY OF ENTOMOPATHOGENIC NEMATODES AGAINST *HETEROTERMES INDICOLA* (ISOPTERA: RHINOTERMITIDAE) IN THE LABORATORY

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Abstract.- Termite, *Heterotermes indicola* (Wasmann) is one of the devastating insect pests certainly causing significant losses in different agricultural crops all over the world. Bio-efficacy of two Entomopathogenic Nematodes (EPNs) species (*Steinernema* sp. and *Heterorhabditis* sp.) in various concentrations *i.e.* 10, 20, 40, 80, 160 individuals per termite, and control consist of water were evaluated against termite workers and soldiers. These six mentioned treatments were replicated ten times by utilizing completely randomized design. Results of the experiments revealed that EPNs @ 160 EPNs/termite were found to be most effective control against termite workers and soldiers. Furthermore, the concentration and time interval (days) positively reported mortality rate of termites. It is suggested that the EPNs has the potential to be incorporated in a biorational management strategy against termites.

Keywords: Entomopathogenic nematodes, *Heterotermes indicola*, *Steinernema* sp., *Heterorhabditis* sp., Termite, mortality

INTRODUCTION

Termites are found throughout Pakistan including Khyber Pakhtunkhwa. They are either soil or wood inhabiting termites (Chaudhry *et al.*, 1972). *Microtermes mycophagous*, *Microtermes obesi*, *Microtermes unicolor*, *Eremotermes paradoxalis* and *Odontotermes obesus* are the species mainly recorded from agro-ecosystems of Pakistan (Ahmed *et al.*, 2004). Termites like *Odontotermes*, *Heterotermes* and *Coptotermes* have been observed to infest and cause damages to apricot, pear, plum, peach, orange and lemon (Salihah *et al.*, 1994).

Many methods and techniques have been practiced for termite control. Amongst these methods, chemicals control method dominating and practice for a long period. Chemicals application is not only expensive for farmer's community but also has negative effect on environment and health of living organisms. The

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insecticides in liquid or dry formulation viz., chlorpyrifos, imidacloprid and fipronil are being applied in furrows at the time of sowing of sugarcane crop (Ahmed *et al.*, 2006). The success of such treatment with insecticides is highly variable. There is need to search for alternates to these insecticides to avoid adverse effects of insecticides. So, other methods erupted interest especially biological control agents for termites control (Grace, 1997).

The EPNs are considered as exceptionally potential agent for insect control and effective bio-control agents having non-polluting properties (Gaugler *et al.*, 1997). EPNs used for the control of major insect pests, which causes serious damage in food and fiber crops. Approximately, hundred different laboratories explored nematodes and their bacterial symbionts in more than 60 countries from every inhabited continent. EPNs have been found in diverse ecological conditions including cultivated fields, forests, grasslands, deserts and ocean beaches (Hominick *et al.*, 1997).

EPNs Steinernematid and Heterorhabditid reported as a group of biological control agents against termites. These EPNs received great attention in the 1990s of the world (Kaya and Gaugler, 1993). Hundreds of different species from most orders of insects were susceptible to various EPNs in laboratory tests. Nematodes have the advantages of being easy to apply, compatible with many pesticides and finding their hosts either actively or passively (Smart, 1995). Termites live and forage in habitats that are moist, cool, and without direct sunlight such as soil or wood materials. These environmental conditions are ideal for the survival and movement of Steinernematid and Heterorhabditid nematodes. Therefore, these nematodes provide the basis for interest in their role in control of subterranean termites.

The present study aims at determining the response of termite workers and soldiers towards different concentrations of two indigenous EPN species i.e. *Steinernema* sp. and *Heterorhabditis* sp. at laboratory conditions.

MATERIALS AND METHODS

Rearing of host (Galleria mellonella L.)

G. mellonella (L.) was reared on artificial diet by mixing 300 ml liquid honey, 400 ml glycerol, 200 ml milk powder, 200 g whole-meal coarse flour, 100 g dried brewer's yeast, 100 g wheat germ and 400 g bran (Ellis *et al.*, 2013). The larvae were collected from honey bee apiaries were placed in a sterilized container having artificial diet along with crumpled paper towel.

The female moths were made to oviposit on crumpled paper towel. The collected larvae started feeding on diet. In the next step, larvae or pupa were harvested and placed in another container, and allowed to pupate or pupa to emerge as adults. The newly appeared adults were mated and females laid eggs. This process is repeated for future generation.

Rearing of entomopathogenic nematode

The EPNs were reared by modifying the method of Lindegren *et al.* (1993). Petri dish of size 9 cm was sterilized in autoclave at 121°C, 15 lbs/inch². It was cooled and then lined with Whatman's filter paper no.1. Suspension of Juvenile 1 EPN (500 IJs/ ml) was dropped in each petri dish having filter paper. Ten of the 5th instars of *G. mellonella* larvae were placed on petri dish and incubated at 20±5°C for 48 hour at (8:16) dark light photoperiod. *G. mellonella* cadavers were transferred to White traps (White, 1929) which consisted of a glass Petri dish (9 cm in a diameter) filled with distilled water to a depth of 0.5 cm (modified). The bottom of an inverted Petri dish (3 cm in a diameter) was placed in the bigger Petri dish. A sheet of filter paper was placed on the smaller Petri dish. Larger Petri dishes filled with distilled water till the filter papers remain moistened. The dead larvae were placed on the filter paper and incubated at room temperature till all the nematodes progeny emerged and moved down into the water. IJs of EPNs were harvested after two weeks and EPNs suspension were then kept in tissue culture flask at 10°C in incubator for further experiments.

The efficacy of EPNs against termite workers and soldiers

This experiment was carried out according to Mankoski *et al.* (2005) who studied response of termite workers and soldiers to different concentrations of EPNs under controlled laboratory condition. Each petri dish provided with 1 ml of water having different concentrations i.e 10, 20, 40, 80 and 160 EPNs / Termite. Control having 1 ml of distilled water. Each petri dish contained 20 termite workers and 08 termite soldiers. The experiment was replicated ten times at completely Randomized Design in a dark room at room temperature. The data recorded as mean mortality of termite worker and soldiers by both species of EPNs. The mean variables were analyzed through LSD test at p 0.5 (Gomez, 1984).

RESULTS

Table I and Figure 1 shows percent mortality of termite worker caused by different concentrations of *Steinernema* sp. Mortality of termite workers was maximum (82.3%) In experiment, treatment of 160 individuals per Termite followed by minimum mortality (12.75%) in experiment treatment of 80 EPNs/Termite (72.2%). Further 62.4, 33.7 and 12.8 % mortality was observed by 40, 20 and 10 EPNs/Termite.

TABLE I. BIO-EFFICACY OF *STEINERNEMA CARPOCAPSAE* (*Se*) AGAINST TERMITE WORKER.

Treatment	TIME (DAYS)						Mean
	1 st	2 nd	3 rd	4 th	5 th	6 th	
30 termites/ml	12.5 p	16.5 o	34.5 n	39.5 m	44.0 kl	55.0 h	12.8 e
40 termites/ml	34.5 n	40.0 m	46.5 jk	50.0 j	56.0 gh	69.0 e	33.7 d
50 termites/ml	43.5 klm	41.0 lm	60.0 f	69.0 e	70.0 e	90.5 c	62.4 c
60 termites/ml	45.5 k	50.5 i	59.0 fg	90.0 c	90.5 c	98.0 ab	72.2 b
160 termites/ml	56.5 gh	62.0 f	84.5 d	94.5 b	96.5 ab	100.0 a	82.3 a
Control	8.5 q	13.5 op	13.5 op	13.5 op	13.5 op	14.0 op	12.75 f
Mean	33.4 e	37.3 d	49.8 c	59.4 b	61.8 b	71.1 a	

Note: Means followed by different letters are significant at 5% level of probability followed by LSD Test

LSD for treatment= 0.7477

LSD for days= 0.7477

LSD treatment* days= 1.8314

Mortality was also significantly affected with time interval. As the time interval increased the mortality rate boosted up. Mortality was lowest (33.4 %) after 1st day while highest (71.1%) was recorded after 6th day.

Table II and Figure 2 show that mortality of termite workers was significantly affected by different concentrations of *Heterorhabditis* sp. Treatments showed that mean mortality of termite workers was maximum (81.2%) after treatment with 160 EPNs/ Termite, while minimum mortality (11.41%) was recorded in control followed by highest mortality (68.9%) recorded in 80 EPNs/ Termite. in addition 40, 20 and 10 EPNs/ Termite showed mortality of 60.3, 51.8 and 28.1%, respectively.

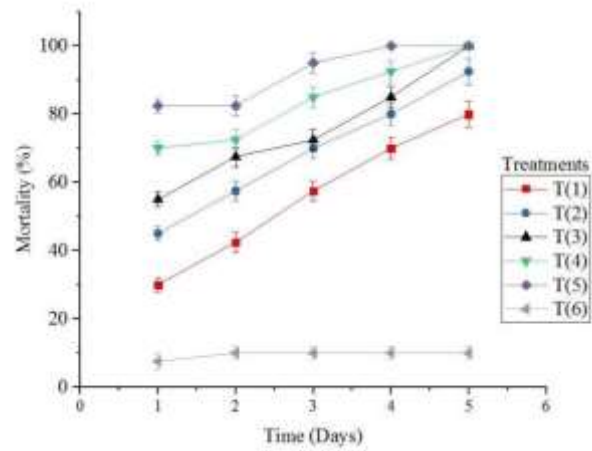
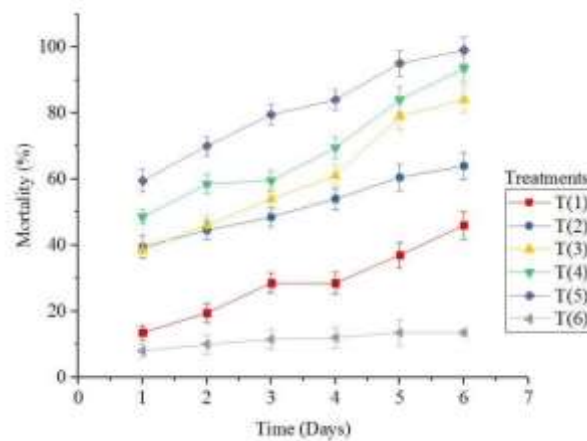
**A****B**

Fig. 1. Effect of *Steinernema* sp. application against termite soldiers (A) and workers (B)

Time interval also plays significant role in percent mortality. The percent mortality increased with increasing time interval. It was recorded lowest (34.5 %) after 1st day whereas it was highest (66.7 %) after 6th day of exposure.

TABLE II. BIO-EFFICACY OF *HETERORHABDITIS BACTERIOPHORA* (Hb) AGAINST TERMITE WORKER.

Treatment	TIME (DAYS)						Mean
	1 st	2 nd	3 rd	4 th	5 th	6 th	
30 termites/ml	13.5 n	19.5 m	28.5 k	28.5 k	37.0 k	46.0 ij	28.0 e
40 termites/ml	39.5 k	44.5 j	48.5 i	54.0 h	60.5 fg	64.0 f	51.8 d
50 termites/ml	38.0 k	46.0 ij	54.0 h	61.0 fg	79.0 d	84.0 c	60.3 c
60 termites/ml	48.5i	58.5 g	59.5 g	69.5 c	84.0 c	93.5 b	68.9 b
160 termites/ml	59.5 g	70.0 e	79.5 d	84.0 c	95.0 b	99.0 a	81.2 a
Control	8.0 p	10.0 op	11.5 op	12.0 o	13.5 o	13.5 o	11.41 f
Mean	34.5 f	41.42 e	46.2 d	51.50 c	61.5 b	66.7 a	

Note: Means followed by different letters are significant at 5% level of probability followed by LSD Test

LSD for interval= 0.7572

LSD for Treatment= 0.7572

LSD for interval* Treatment= 1.8547

Table III shows morality of increasing termite soldiers after exposure to different concentrations of EPNs (*Steinernema* sp.). The mean mortality of termite workers was maximum (95.5%) in 160 EPNs/ Termite and minimum (7.5%) in control followed by 80 EPNs/Termite (89.0 %). In addition, 40, 20 and 10 EPNs/ Termite caused 80, 70 and 56% mortality, respectively. Mortality also increased significantly by increasing time interval. Mortality was lowest (33.4 %) after 1st day, while it was highest (81.67%) after 6th day.

TABLE III. BIO-EFFICACY OF *STEINERNEMA CARPOCAPSAE* (Sc) AGAINST TERMITE SOLDIERS.

Treatment	TIME (DAYS)						Mean
	1 st	2 nd	3 rd	4 th	5 th		
30 termites/ml	27.5 f	42.5 e	57.5 d	70.0 c	82.5 b	56.0 e	
40 termites/ml	42.5 e	55.0 d	70.0 c	82.5 b	100 a	70.0 d	
50 termites/ml	67.5 c	55.0 d	82.5 b	95.0 a	100 a	80.0 c	
60 termites/ml	67.5 c	82.5 b	95.0 a	100 a	100 a	89.0 b	
160 termites/ml	95 ab	100 a	100 a	100 a	100 a	95.5 a	
Control	7.5 g	7.5 g	7.5 g	7.5 g	7.5 a	7.5 f	
Mean	49.17 e	56.25 d	68.75 c	75.83 b	81.67 g		

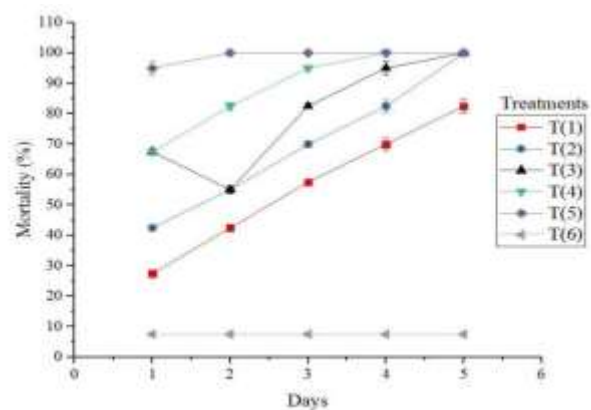
Note: Means followed by different letters are significant at 5% level of probability followed by LSD Test

LSD for days= 1.8188

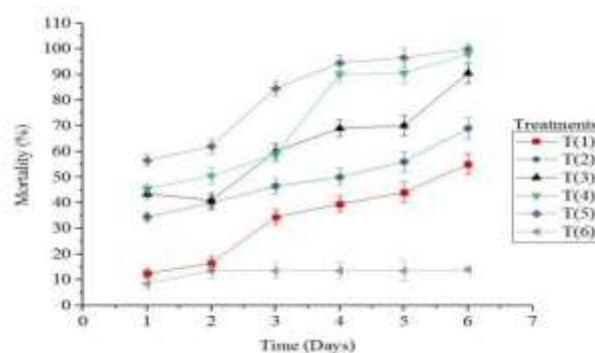
LSD for Treatment= 1.9924

LSD for days* Treatment= 4.4552

Table IV reported morality of termite soldiers was significantly affected by different concentrations of *Heterorhabditis* sp. Results of Table IV showed that mean mortality of termite soldiers was maximum (92%) in 160 EPNs/ Termite, Whereas minimum (9.5%) mortality was informed in control, followed by maximum (84 %) mortality recorded in 60 EPNs/ Termite. Moreover, 40, 20 and 10 EPNs/ Termite gave the mortality rate of 76, 69 and 56%, respectively.



A



B

Fig. 2. Effect of *Steinernema* sp. on termite soldiers (A) and workers (B)

TABLE IV. BIO-EFFICACY OF *HETERORHABDITIS BACTERIOPHORA* (Hb) AGAINST TERMITE SOLDIERS.

Treatment	TIME (DAYS)					Mean
	1 st	2 nd	3 rd	4 th	5 th	
30 termites/ml	30 h	42.5 g	57.5 f	70.0 e	80.0 cd	56.0 e
40 termites/ml	45.0 g	57.5 f	70.0 e	80.0 cd	92.5 ab	69.0 d
50 termites/ml	55.0f	67.5 e	72.5 de	85.0 bc	100 a	76.0 c
60 termites/ml	70.0 e	72.5 de	85.0 bc	92.5 ab	100 a	84.0 d=b
160 termites/ml	82.5 c	82.5 c	95.0 a	100.0 a	100 a	92.0 a
Control	7.5 i	10.0 i	10.0 i	10.0 i	10.0 i	9.5 f
Mean	48.3 e	55.42 d	65.0 c	72.92 b	80.42 a	

Note: Means followed by different letters are significant at 5% level of probability followed by LSD Test

LSD for Interval= 2.0242

LSD for Treatment= 2.2174

LSD for Interval*Treatment= 4.9582

Meanwhile, mortality was recorded significantly with reference to time. Mortality was lowest (48.3 %) after 1st day and highest (80.42%) after 6th day.

DISCUSSION

The nematode infectivity test showed that different nematodes species had very different levels of infectivity against termites. *Steinernema* sp. was found to be more effective than *Heterorhabditis* sp. in term of virulence. *Steinernema* sp. was the better of the two species tested in terms of virulence. Danthanarayana and Vitarana (1987) achieved effective control against a dry-wood termite, *G. dilatatus* through *Heterorhabditis* sp. to protect tea bushes in field conditions.

Quite often, programmes aiming at the development of strategies for the biological control of insects start with laboratory bioassays. These assays screen local EPNs for various beneficial traits in order to identify potential biocontrol candidates for pest control and to reduce the number of strains or species that need to be tested in the field (Patterson Stark and Lacey, 1999; Shapiro and McCoy, 2000). The results of present bioassays indicated that isolates of both *Steinernema* sp. and *Heterorhabditis* sp. exhibited cruiser and ambusher type of insect search strategy. Our findings on relative Entomopathogenic nematode longevity are consistent with some previous laboratory studies but not with

others. For example, our findings of superior survival of *Steinernema* sp. relative to *Heterorhabditis* sp. are similar to those of Kung *et al.* (1990). Also consistent with our study, Molyneux (1985) reported greater longevity in *S. carpocapsae* and *S. glaseri* relative to two *Heterorhabditis* spp. but, unlike our study and that of Kung *et al.* (1990), survival of *S. glaseri* in Molyneux's study was greater than that of *S. carpocapsae*. One possible cause for these discrepancies is strain variation used in each study.

Results regarding invasion time of Entomopathogenic nematodes also showed significant differences among selected isolates. This is in agreement with Glazer (1992) who revealed a large variability in invasion capacities among different nematode isolates, suggesting that measuring invasion time could be a useful strategy for the selection of virulent strains of EPNs. Hominick and Reid (1990) proposed the use of invasion efficiency as a direct measure of nematode infectivity. These authors assumed that the nematodes with the greatest efficacy against a target insect would have the highest invasion efficiency. In this respect, based on their lowest values of penetration mortality 28.1% (6th day of exposure) and their highest penetration rates and mortality (82.3%) 6th day of exposure, *Steinernema* sp. could be the most virulent among the tested species. Significant differences between species were observed with respect to the lethal time showing that EPNs exhibited time-dependent susceptibility. This suggests that insects parasitized with a higher number of nematodes take a shorter time to die. High infection rates increase toxins produced by developing nematodes (Burman, 1982) and their symbiotic bacteria (Akhurst and Boemaere, 1990).

Nematode concentration and incubation time had significant effects on the mortality. However, Yu *et al.* (2010) compared virulence of three novel strains of *S. riobrave* (3-8b, 7-12, and TP) against subterranean termites *H. aureus*, *R. flavipes*, and *C. formosanus* workers. It was found that *H. aureus* was very susceptible to all the *S. riobrave* strains and termites in all nematode treatments were dead after 4 days.

It was found that highest mortalities in *H. indicola* caused by *Steinernema* sp. Shahina and Tabassum (2010) found similar results in filter paper and sand assay. Mankowski *et al.* (2005) examined the attachment and infectivity of two EPN species, *S. carpocapsae* and *H. indica*, on soldiers and workers in two subterranean termite species, *C. formosanus* and *C. vastator*. In attachment tests with *S. carpocapsae*, they noticed that more nematodes attached to soldiers of *C. formosanus* and *C. vastator*. When soldiers alone or workers alone are exposed to

the nematodes, there is a differential susceptibility of soldiers and workers to nematode infection with soldiers being more susceptible than workers. The reason for this differential response to nematode infection could be due to the soldiers grooming behavior. The bioagent *S. carpocapsae* was noticed more effective for control of the termites, but termite soldiers was highly susceptible than termite workers. Overall, the mortality increased as the nematode concentrations increased and vice versa.

In general the results showed that both tested EPNs (*Steinernema* sp., *Heterorhabditis* sp.) were pathogenic to the (*H. indicola*) a termite pest. However, there are many other biological factors that can affect the final choice of the tested EPN species/isolates for the control of termites. Abiotic factors, such as UV radiation, fluctuating soil temperature and moisture content, and antagonists in the field could prevent the EPNs from realizing their full potential as bio-insecticides (Gray, 1988; Grewal, 2011). Further studies in field-simulated conditions in the laboratory, in glasshouses and in the field are still needed before making a choice of the promising isolates for biological control of termite.

CONCLUSION AND RECOMMENDATIONS

It is concluded from our findings that both of our tested EPNs i-e *Steinernema* sp., *Heterorhabditis* sp. showed promising results against subterranean termite (*H. indicola*) workers as well as soldiers. So it is suggested from our finding that EPNs could be used as a part of IPM strategy against termite for a safe and better control.

Contribution of Authors

ZU collected primary data, analyzed the data and compiled this article. AURS was the major supervisor and FA was Co- Supervisor FK guided in rearing and data collection. AU helped in data analysis and paper write up.

REFERENCES

- AHMED, S., AKBAR, W. AND RIAZ, A. M., 2004. Effect of crop rotation and intercropping on subterranean termites in wheat at Faisalabad. *Pakistan J. Ent.*, **26**: 25-30.
- AHMED, S., MUSTAFA, T., RIAZ, M. A. AND HUSSAIN, A., 2006. Efficacy of insecticides on subterranean termites in sugarcane at bhakkar. *Int. J. Agric. Biol.*, **8**: 508–10.
- AKHURST, R. J. AND BOEMARE, N. E., 1990. Biology and taxonomy of *Xenorhabdus*. In: (eds. R. Gaugler, & H.K. Kaya,). *Entomopathogenic nematodes in biological control*. Boca Raton, Florida, CRC Press, pp. 75– 90.

- CHAUDRY, M. I., AHMAD, M. MALIK, N. K. AKHTAR, M. S. AND ARSHAD, M., 1972. *Termites of Pakistan: Identify distribution, and ecological relationship*. Final Technical Report. PL-480 Project No. A17-fs-12. Peshawar., 70pp.
- DANTHANARAYANA, W. AND VITARANA, S., 1987. Control of the live-wood tea termite *Glyptotermes dilatatus* using *Heterorhabditis* sp. (Nemat.). *Agric. Ecosys. Environ.*, **19**: 333 – 342.
- ELLIS, J. D., GRAHAM, J. R. AND MORTENSEN, A., 2013. Standard methods for wax moth research. *J. Apicult. Res.* **52**: DOI 10.3896/IBRA.1.52.1.10
- GAUGLER, R., WILSON, M. AND SHEARER, P., 1997. Field release and environmental fate of a transgenic entomopathogenic nematode. *J. Biol. Contr.*, **9**: 75–80.
- GLAZER, I., 1992. Invasion rate as a measure of infectivity of steinernematid and heterorhabditid nematodes to insects. *J. Inverteb. Pathol.*, **59**: 90–94.
- GOMEZ, S., DENHOLM, I. AND DEVONSHIRE, A., 2000. The ups and down of insecticide resistance in peach potato aphid (*Myzus persicae*) in the UK. *Crop Protec.*, **19**: 873879.
- GRACE, J. K., 1997. Biological control strategies for suppression of termites. *J. agric. Ent.*, **14**: 281-289.
- GRAY, N. F., 1988. Ecology of nematophagous fungi-effect of the soil nutrients N, P and K, and 7 major metals on distribution. *Pl. Soil*, **108**: 286– 290.
- GREWAL, P. S., EHLERS, R.U. AND SHAPIRO- ILAN, D. I., 2005. *Nematodes as biological control agents*. CAB International Publishing, Wallingford.
- HOMINICK, W.M. AND REID, A.P., 1990. Perspectives on entomopathogenic nematology. In: *Entomopath nematodes in biological control* (eds. R. Gaugler, and H. K. Kaya). CRC, Boca Raton, FL. pp. 327–345.
- HOMINICK, W.M., BRISCOE, B.R., DEL PINO, F. G., HENG, J. A., HUNT, D. J., KOZODOY, E., MRAC[~] EK, Z., NGUYEN, K. B., REID, A. P., SPIRIDONOV, S., STOCK, S.P., STURHAN, D., WATURU, C. AND YOSHIDA, M., 1997. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. *J. Helminthol.*, **71**: 271-298.
- KAYA, H. K. AND STOCK, P. S., 1997. Techniques in insect nematology. In: *Manual of techniques in insect pathology* (ed. L. A. Lacey). Academic Press, San Diego, CA, pp. 281– 324.
- KUNG, S., GAUGLER, R. AND KAYA, H. K., 1990. Soil type and Entomopathogenic nematode persistence. *J. Inverteb. Pathol.*, **55**: 401–406.
- LINDEGREN, J. E., VALERO, A. K. AND MACKEY, E. B., 1993. Simple in vivo production and storage methods for *Steinernema carpocapsae* infective juveniles. *J. Nematol.*, **25**: 193–197.
- MANKOWSKI, M. E., KAYA, H. K., GRACE, J. K. AND SPIES, B., 2005. Differential susceptibility of subterranean termite caste to entomopathogenic nematodes. *Biocont. Sci. Technol.*, **15**: 367-377.
- MAULDIN, J. K. AND BEAL, R. H., 1989. Entomogenous nematodes for control of subterranean termites, *Reticulitermes* spp. *J. econ. Ent.*, **82**: 1638 –1642.
- MCCOY, C.W., SHAPIRO D.I., DUNCAN, L.W., AND NGUYEN, K., 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *Biol. Contr.*, **19**: 182–190. 10.1006/bcon.2000.0852

- MOLYNEUX, A.S., 1985. Survival of infective juveniles of *Heterorhabditis* spp., and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. *Rev. Nematol.*, **8**: 165–170.
- PATTERSON, S.J.E. AND LACEY, L.A., 1999. Susceptibility of western cherry fruit fly (Diptera: Tephritidae) to five species of entomopathogenic nematodes in laboratory studies. *J. Inverteb. Pathol.*, **74**: 206–208.
- SALIHAI, Z., SATTAR, A. AND KHATOON, R., 1994. Detection of nesting system of termites (*Heterotermes indicola*) at NIFA campus. NIFA Annual Report. NIFA Ann. Rep. 1993-94: 96-104.
- SHAHINA, F. AND TABASSUM, K.A., 2010. Virulence of *Steinernema pakistanense* against different insect species in laboratory condition. *Pak. J. Nematol.*, **28**: 279–284.
- SHAPIRO, D.I., CATE, J.R., PENA, J., HUNSBERGER, A. AND MCCOY, C.W., 1999. Effects of temperature and host range on suppression of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) by entomopathogenic nematodes. *J. econ. Ent.*, **92**: 1086–1092.
- SHIELDS, E. J., TESTA, A., MILLER, J. M. AND FLANDERS, K. L., 1999. Field efficacy and persistence of the entomopathogenic nematodes *Heterorhabditis bacteriophora* 'Oswego' and *H. bacteriophora* 'NC' on Alfalfa snout beetle larvae (Coleoptera: Curculionidae). *Environ Ent.*, **28**: 128–136.
- SMART, G. C., 1995. Entomopathogenic nematodes for the biological control of insects. *J. Nematol.*, **27**: 529-534.
- WOODRING, J. L. AND KAYA, H.K., 1988. Steinernematid and Heterorhabditid nematodes .A handbook of biology and techniques. *South. Cooper. Ser. Bull.*, **331**: p. 1-30.
- YU, H., GOUGE, D.H AND SHAPIRO-ILAN, D.I., 2010. A novel strain of *Steinernemariobrave* (Rhabditida: Steinernematidae) possesses superior virulence to subterranean termites (Isoptera: Rhinotermitidae). *J. Nematol.*, **42**: 91–95.

BACTERIAL INOCULATION AFFECTS INTERNAL ANATOMY OF *TRITICUM AESTIVUM* L.

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Abstract.- Plant growth promoting rhizospheric bacteria have been found to affect the plants beneficially. These rhizospheric bacteria use different mechanisms to trigger the growth of plants. In the current study, auxin producing *Cronobacter* (AL2) and *Enterobacter* (A12G) spp. were treated with *Triticumaestivum* L. var.FD-08 and the inoculated and control plants were studied to investigate the effect of bacterial treatment on the growth of plants. For this purpose, growth and biochemical parameters as well as anatomical parameters of treated and non-treated plants were studied. The pronounced increase in different growth parameters of inoculated plants were observed. Biochemical analysis of different inoculated plants has also shown increase in auxin, protein, chlorophyll a, b, total chlorophyll and stem anatomy of treated and non-treated seedlings was also studied. Microscopic observations revealed that the selected auxin producing bacterial strains affected the anatomical features of root and stem of the inoculated seedlings positively as compared to the non-inoculated seedlings. Thus these auxin producing bacterial strains can be used as biofertilizers to enhance crop yield.

Keywords: PGPR, PMI, root hair, *Triticum aestivum*.

INTRODUCTION

Plant growth promoting rhizobacteria have also been observed in the enhancement of plant growth by providing various promotive substances and facilitating nutrient uptake from the environment (Meng *et al.*, 2016). Plant growth promoting bacteria are used to improve the yield of crop in the agricultural area (Papenfus *et al.*, 2015). PGPRs has great influence on the plants growth by indirect or direct means. Direct mechanisms possess the biological nitrogen fixation, phosphorus utilization, iron acquisition, hormone production while the indirect mechanisms include induced systemic resistance (ISR), nutrients competition, antibiotics, excretion of lytic enzymes, toxins excretion and metabolites productions including HCN and siderophores (Jha *et al.*, 2015). The atmospheric nitrogen is not accessible for the plants. Hence, rhizobacteria fix the atmospheric nitrogen into ammonia (Souza *et al.*, 2015). PGPR were also observed to solubilize the tri-calcium type phosphates in the soil medium by the secretion of organic acids (Rout *et al.*, 2014). The bacteria producing siderophores enhance the plants iron utilizations in iron-restricted environments

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(Asari *et al.*, 2015). Phytohormones are the chemical messengers, usually synthesized in particular parts of the plants and translocated to another part where it is needed to perform a physiological process (Asari *et al.*, 2015). The phytohormones include auxin, cytokinins and gibberellins etc. They increase the growth of plant bodies (Akhtar *et al.*, 2012). Among the group of auxin, a phytohormone involved in various long term and short term responses of plant growth. The long term responses includes, cell division and differentiation, while the short term responses includes, cell elongation (Goswami *et al.*, 2016). The induction of seed germination and the emergence, leaf growth and stem development are stimulated by the gibberellin production in plants (Vejan *et al.*, 2016). PGPR possess an enzyme named as ACC deaminase that is capable of decreasing ethylene level and facilitating plant growths. Cytokinins show many positive physiological effects such as division of cell, development of root, formations of root hairs and shoot stimulation (Goswami *et al.*, 2016). Alongwith direct mechanisms, there are also indirect mechanisms in PGPR. In one of these mechanisms, the microbes live outside the plant body and resists the plants from pathogens attack. Similarly the PGPR sometimes produce an immunity against other bacteria, fungi or viruses which are pathogenic in nature and cause damaging effect on the plants. This process ISR (Ahemad and Kibrat, 2014). Exopolysaccharides production is an important characteristics of bacteria. It helps in colonization to any surface (Jha *et al.*, 2015). The current study aims to analyse the impact of auxin-producing bacteria on growth of *Triticum aestivum*. Plants were inoculated with two identified strains *Enterobacter* sp. and *Cronobacter* sp. Growth parameters and biochemical contents of both plants were analyzed. Bacterial inoculated seeds of *Triticum aestivum* were also grown under lab conditions and seedlings were observed after growth where significant enhancements in shoot length and root system architecture of treated seedlings were observed in comparison with the non-treated seedlings. Root and stem anatomy of the treated and non-treated seedlings were studied microscopically using thin root and stem sections. The auxin production potential of bacterial isolates was also analyzed.

MATERIALS AND METHODS

Bacterial growth conditions

Two already isolated and identified bacterial strains by Ahmed and Hasnain (2010) *i.e.*, *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G) were used in the present study. All the bacterial strains were routinely grown using L-Agar and L-Broth medium at 37 °C for 24 h.

Plant microbe interaction

Seeds were sterilized by using detergent and 0.1% HgCl₂. Bacterial cultures were grown in LB broth in test tubes for 24 h at 37°C. The seeds were incubated in bacterial inoculum for 40 min, after that they were sown in pots. After 25-30 days, wheat seedlings were harvested and growth parameters were measured. Soluble protein estimation was carried out by using Lowry *et al.* (1951) method. The absorbance of pure sample was observed at 750nm in Beckman D-2 spectrophotometer. Soluble protein content was estimated by standard curve. Auxin estimation was performed according to Mahadevan *et al.* (1984). Optical density of pure IAA sample was taken at 535nm. Auxin content was calculated using IAA standard curve. Chlorophyll content was estimated by using the method of Wellburn (1994). The absorbance of the green solution was estimated at 663 and 645 nm. Total chlorophyll was estimated according to the following formula:

$$\begin{aligned}\text{Chlorophyll a (mg g}^{-1}\text{)} &= (12.72 A_{663} - 2.59 A_{645}) \times V/(m \times 1000) \\ \text{Chlorophyll b (mg g}^{-1}\text{)} &= (22.88 A_{663} - 4.67 A_{645}) \times V/(m \times 1000) \\ \text{Total chlorophyll content} &= \text{Chl a} + \text{Chl b}\end{aligned}$$

Anatomical studies

Seeds of *Triticum aestivum* were inoculated with each isolate separately in medium sized petri dishes on two layers of sterilized moistened filter paper. After seven days, root length, number of lateral and primary roots were observed and recorded. Section cutting of root and stem was carried out according to the Afry *et al.* (2012) to observe various anatomical features of inoculated and non-inoculated *Triticum aestivum* seedlings.

RESULTS*Plant microbe interaction*

The selected auxin-producing bacteria predominantly influence all the growth parameters. Significant enhancement in the percentage of germination was estimated by the treatment of bacterial isolates *Enterobacter* sp. (A12G) and *Cronobacter* sp. (AL2) upto 50 and 16% respectively, in comparison to non-inoculated plants while maximum enhancement in shoot length was observed with bacterial strain *Cronobacter* sp. (AL2) (19%) followed by

Enterobacter sp. (A12G) 18% in comparison to the non-treated plants. The maximum enhancement in length of root was shown by bacterial strain *Enterobacter* sp. (A12G) i.e., 91% in comparison to the non treated plants while maximum enhancement in the number of leaves were observed through the plants treated with bacterial strain *Enterobacter* sp. (A12G) upto 4% respectively, in comparison to control plants. Although, the plants treated with *Enterobacter* sp. (A12G) showed maximum enhancement in fresh weight upto 14% in comparison with the control plants (Fig. 1). Auxin content, protein content and chlorophyll content of treated and control plants were also estimated. The plants inoculated with the bacterial strains *Enterobacter* sp. (A12G) exhibited pronounced enhancements in the auxin amount upto 188% as compared to control plants. PGPR exert prominent effect on the protein content of plants. Among the isolates, enhancement in protein content was exhibited by the plants treated with strain *Enterobacter* sp. (A12G) (20%) in comparison to control plants (Fig. 2).

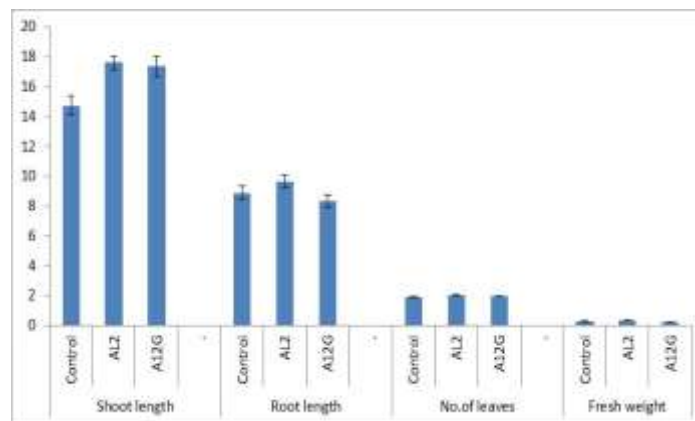


Fig. 1. Effect of bacterial inoculations [*Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G)] on shoot length (cm), root length (cm), number of leaves and fresh weight (g) of *Triticum aestivum* L.

Prominent effects of PGPR were also observed on the chlorophyll 'a' and chlorophyll 'b' content of inoculated plants in comparison to the control plants. The plants inoculated with *Enterobacter* sp. (A12G) exhibited enhancement in chlorophyll 'a' content upto 44% 14 as compared to non-inoculated plants while chlorophyll 'b' content showed enhancement in the plants treated with bacterial strain *Enterobacter* sp. (A12G) (22%) in comparison to non inoculated plants (Fig.3). The maximum increase in total chlorophyll content was recorded

by the bacterial isolate *Enterobacter* sp. (A12G) (7%) as compared to control plants.

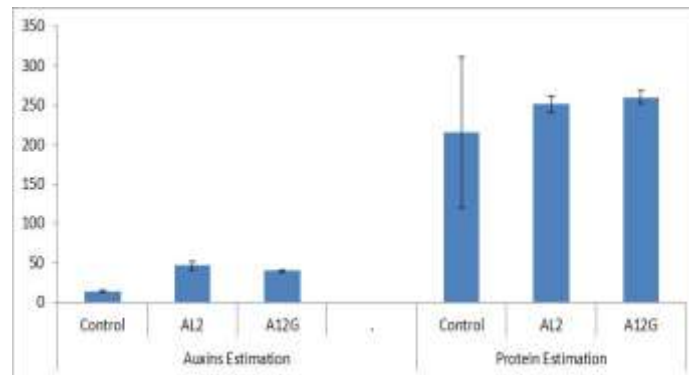


Fig. 2. Effect of bacterial inoculations *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G)] on auxin content (µg/g) and protein content (µg/g) of *Triticum aestivum* L.

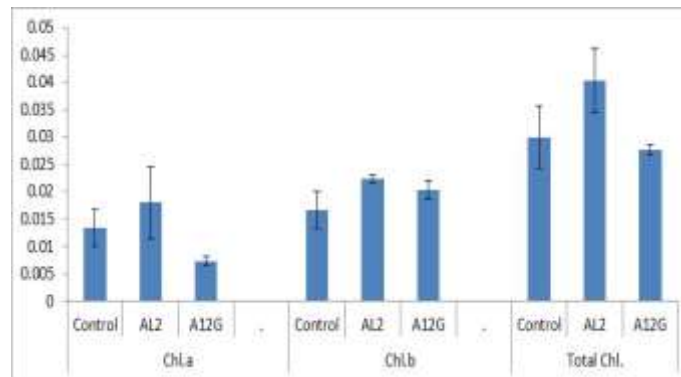


Fig. 3. Effects of bacterial inoculations *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G)] on chlorophyll content (µg/g) of *Triticum aestivum* L.

Root system architecture

PGPR performs significant role in development of root system architecture *i.e.*, root length, number of primary roots and number of lateral roots. Maximum enhancement in root length was observed by *Cronobacter* sp. (AL2) upto 73%, respectively, in comparison to the control seedlings. The plants

inoculated with the microbial strain *Cronobacter* sp. (AL2) exhibited maximum enhancement in number of primary roots upto 57% as compared to the non-treated seedlings while significant enhancement in the number of secondary roots, in comparison to non treated seedlings was exhibited by the bacterial strain. Moderate enhancements in number of secondary roots were exhibited with the bacterial strains *Cronobacter* sp. (AL2) i.e., 566 %, as compared to the control seedlings. Bacterial strain *Cronobacter* sp. (AL2) produced 80 root hairs and exhibited maximum enhancement in root hair number, as compared to the non-inoculated control seedlings which have 21 root hairs while the bacterial strain *Cronobacter* sp. (AL2) exhibited maximum enhancement in root hairs length i.e., 200% respectively, as compared to the non-inoculated control seedlings (Table I). Bacterial strain *Enterobacter* sp. (A12G) showed thickened epidermal cells as compared to the control seedlings. In relation to the cortex region, thickness in cortex area upto 5-cells was observed by the application of bacterial strain *Enterobacter* sp. (A12G) in comparison to the non treated seedlings. The treatment of bacterial strain *Cronobacter* sp. (AL2) enhanced the size of endodermal cells upto 50% as compared to the non-inoculated seedlings while the bacterial isolates *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G) showed enhancement in the thickness of pericycle cells as compared to the control seedling (Fig. 4). The bacterial strains *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G) and produced seven bundles of metaxylem as compared to the control seedlings which have five bundles of metaxylem while the bacterial strain produced five bundles of protoxylem as compared to the control seedlings having four protoxylem bundles. In relation to the phloem diameter, application of bacterial strains *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G) produced wider diameter of phloem bundles in comparison to non-inoculated seedlings. Although, the bacterial strains *Cronobacter* sp. (AL2), exhibited wider diameter of ground tissue as compared to the control seedlings.

TABLE I.- ANATOMICAL STUDY OF TREATED AND NON-TREATED PLANTS OF *TRITICUM AESTIVUM* L. (ROOT)

Strains	No. of root hair	Length of root hair	Ground Tissue	Endodermis	Vascular Bundles
Control	21	Short	Thin	Thin	05
<i>Enterobacter</i> sp. (A12G)	80	Long	Thick	Thick	07
<i>Cronobacter</i> sp. (AL2)	80	Long	thick	Thick	07



Fig. 4. Stem anatomy of *Triticum aestivum* A- non-inoculated and B- inoculated [*Cronobacter* sp. (AL2)]

TABLE II.- ANATOMICAL STUDY OF TREATED AND NON-TREATED PLANTS OF *TRITICUM AESTIVUM* L. (STEM)

Strains	No. of Trichomes	Epidermis	Hypodermis	Vascular Bundles
Control	40	Thin	Thin	18
<i>Enterobacter</i> sp. (A12G)	55	Thick	Thick	21
<i>Cronobacter</i> sp. (AL2)	50	Thick	Thick	25

Stem system architecture

PGPR exhibited enhancements in the length as well as number of trichomes in inoculated wheat seedlings, in comparison with the non-treated seedlings. In relation to the trichome number, bacterial strains *Enterobacter* sp. (A12G) produced 48 trichomes and exhibited maximum enhancement in trichome numbers, as compared to the non-inoculated control seedlings which have 40 trichomes (Table II). In addition to the trichome number, strain *Cronobacter* sp. (AL2) exhibited enhancement in trichomes length *i.e.*, 200% in comparison to control seedlings. The application of *Cronobacter* sp. (AL2) exhibited significantly thickened epidermal as well as hypodermal cells as compared to the control seedlings. Maximum enhancement in the number of scattered vascular bundles *i.e.*, 23, was observed by application of *Enterobacter* sp. (A12G) compared to control.

DISCUSSION

In plant-microbe studies, various growth parameters analyzed were germination percentage, shoot and root length. All of these parameters showed increase in the development of *T. aestivum* L. treated with PGPR1 as compared with the control plants. The maximum increase in the percentage of seed germination upto 50% was recorded in plants treated with bacterial isolate *Enterobacter* sp. (A12G), in comparison to non treated plants while the plants inoculated with bacterial strain *Enterobacter* sp. (A12G) resulted in significant enhancement in the root length upto 9% in comparison to the non-treated plants. Seed treatment with growth enhancing rhizobacterial strains improved seed germination in *T. aestivum*, due to the increased biosynthesis of phytohormones like gibberellins, which triggered the activity of specialized enzymes that enhanced early seed germination, such as α -amylase and other specific enzymes like nuclease and protease which brought an increase in the hydrolysis and availability of starch accumulation (Mangmang *et al.*, 2014). Besides, significant enhancement in seedling growth and development is also due to the better synthesis of auxin which improves cell growth (Abiala *et al.*, 2015). The rhizobacterial IAA also enhances root exudation by loosening the root cell walls which in turn helps in colonization of rhizobacteria and growth of roots. PGPR may also promote plant growth through iron acquisition for plants by the production of siderophores, nitrogen fixation, phytohormones secretion and decrease in the ethylene levels of plants by 1- amino cyclopropane 1-carboxylate deaminase activity of PGPR (Goswami *et al.*, 2016). Our results correlate with the findings of Meena and her coworkers (2016). They reported that inoculated plants of *T. aestivum* with bacterial isolates provide significant positive impacts on the germination of seeds of inoculated plants in comparison to control plant (Meena *et al.*, 2016). Wu and his coworkers (2015) also observed enhanced seed germination, improved shoot and root length, and plant biomass (plant fresh and dry weight) in plants inoculated with growth promoting bacterial strains over control plants (Wu *et al.*, 2015).

Results obtained about auxin content showed that the plants inoculated with the bacterial strain *Enterobacter* sp. (A12G) exhibited maximum significant enhancements in the auxin content upto 188% as compared to control plants. Earlier researches also reported *Zea mays* seeds with inoculated growth promoting bacterial isolates and found to produce 60 % enhancement in indole acetic acid content in comparison to control plants (Noumavo *et al.*, 2015). IAA-producing bacteria also shows beneficial impact on the protein contents of plants.

The PGPR are also called biological nitrogen fixers due to the capability of atmospheric nitrogen fixation (Asari *et al.*, 2015). Thus, making it available for plants and this enhanced nitrogen rate can be utilized for the biosynthesis of amino acid. These nitrogen elements then initiate the accumulation of seed crude proteins. The plants treated with the microbial isolates *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G) resulted in maximum significant enhancement in chlorophyll 'a' content upto 44% in comparison to the control plants. Similarly, enhanced chlorophyll 'b' content was recorded in the plants inoculated with bacterial strains *Cronobacter* sp. (AL2) in comparison to non-inoculated plants while bacterial isolates. IAA-producing rhizobacteria also improve root development and growth and further plant nutrient uptake, thus stimulating indirectly the photosynthetic process. Nitrogen fixation is an important process which enhances the chlorophyll content and thus photosynthetic activity in plants.

PGPR were observed to improve the root system architecture including root length, number of primary and lateral roots. The plants inoculated with the bacterial strains *Cronobacter* sp. (AL2) observed maximum increase in the number of primary roots upto 57 % as compared to the non-treated seedlings while increase in the number of lateral roots was exhibited by microbial strain *Enterobacter* sp. (A12G) *i.e.*, 14%. Application of the selected bacterial strains also exhibited enhanced anatomical features of *T. aestivum* stem in treated seedlings as compared to the control seedling. IAA released from auxin producing bacteria is involved in the enhancement of phloem strands and phloem anastomoses and cell differentiation in xylem bundles of stem at different concentration. Thus, diameter of vascular bundle is enhanced due to auxin production in plants stem. Improvement in the diameter of vascular bundle facilitates plants in the translocation of mineral elements (Rego *et al.*, 2014). Belal and his coworkers also studied the impact of PGPR on the anatomical features of stem of coriander (*Coriandrum sativum* L.). They observed increased thickness in xylem and phloem bundles (Hegazi *et al.*, 2015).

Bacterial strains *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G) exhibited better stem anatomical features of treated plants as compared to non-treated plants. IAA was implicated in the regulation of various biological processes including cell elongation, differentiation, elongation of roots (Menget *et al.*, 2016). Thus IAA revealed to be a growth stimulator of roots; it is produced in apices of stem and then transported towards the lower region in roots of plants and starts to promote the root of growth improving the anatomical features of

roots. Auxin increases cell division in root hairs, thus increases the number and length of root hairs. Increased root hairs promote the absorption of mineral nutrients and water in the plants (Afry *et al.*, 2012). Indolic compounds also increase the thickness of endodermis, cortex, pericycle, phloem and ground tissues in roots. IAA also speed up the process of cell division in endodermal cells and xylem bundles *i.e.*, metaxylem and protoxylem. Thus the size, number and diameters of endodermal cells, cortical cells and xylem bundles were also improved. Increase in cortical cells due to enhanced IAA production in treated plants by PGPR increase the food storage in plants because the cortex cells are specialized for food storage. Increase in cell division of pericycle region promotes the thickness of pericycle layer. Improved metaxylem pays enhancement impact in formation of lateral roots. Xylem elements maintain water conduction (Rego *et al.*, 2014).

CONCLUSION

Generally, the bacterial isolates *Cronobacter* sp. (AL2) and *Enterobacter* sp. (A12G) performed well in improving plant growth in *T. aestivum* where *Enterobacter* sp. (A12G) performed more efficiently than *Cronobacter* sp. Thus, the use of these bacterial strains offers a way to reduce the usage of chemical fertilizers and one step forward to organic farming. The results obtained provide further evidence, concerning rhizobacterial importance as a natural plant growth promotional tool and indicate the potential of exploiting some of these auxin-producing bacteria to further improve crop seedling emergence and establishment. The utilization of these bacterial strains offers an important way to replace and reduce the addition of chemical fertilizers, thereby, reducing environmental pollution which is causing greater harms to mankind and ecosystem.

ACKNOWLEDGEMENT

This work is supported by the financial grant from the University of the Punjab, Lahore, Pakistan.

AUTHORS CONTRIBUTION

The experimental work was carried out by AAG and SW. AA designed and supervised the current research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- ABIALA, M. A., ODEBODE, A.C, HSU, S. F. AND BLACKWOOD, C. B., 2015. Phytobeneficial properties of bacteria isolated from the rhizosphere of maize in southwestern Nigerian soils. *Am. Soc. Microbiol.*, **81**: 4736–4743.
- AFRY, M. M. E., NADY, M. F. E. AND ABDELMONTELEB, E. B., 2012. Anatomical studies on drought-stressed wheat plants (*Triticum aestivum* L.) treated with some bacterial strains. *Acta Biol. Szegediensis.*, **56**:165-174.
- AHEMAD, M. AND KIBRET, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *J. King Saud Univ. Sci.*, **26**: 1-20.
- AHMAD, I., AKHTAR, M. J., ASGHAR, H. N., GHAFOR, U. AND SHAHID, M. 2016. Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake. *J. Pl. Growth Regul.*, **35**: 303–315.
- AHMED, A. AND HASNAIN, S., 2014. Auxins as one of the factors of plant growth improvement by plant growth promoting rhizobacteria. *Polish J. Microbiol.*, **63**: 261-266.
- AKHTAR, A., HISAMUDDIN, ROBAB, M. I. AND ABBASI, SHARF, R., 2012. Plant growth promoting rhizobacteria: an overview. *Scholars Res. Lib.*, **2**: 19-31.
- ASARI, S., TARKOWSKA, D., ROLCIK, J., NOVAK, O., PALMERO, D. V., BEJAI, S. AND MEIJER, J., 2015. Analysis of plant growth-promoting properties of *Bacillus amyloliquefaciens* UCMB5113 using *Arabidopsis thaliana* as host plant. *Planta*, (doi:10.1007/s00425-016-2580-9).
- BARAT, S. P., GUPTA, A., SINGH, D., SRIVASTAV, A. S., KUMAR, R., SINGH, P., FIRDOUS, N. AND SONI, N., 2016. Production of liquid bio fertilizer by using *Azotobacter* Species and their effect on plant growth. *Int. J. Curr. Microbiol. appl.Sci.*, **5**: 654-659.
- CAKMAKCI, R., 2016. Screening of multi-trait rhizobacteria for improving the growth, enzyme activities, and nutrient uptake of tea (*Camellia sinensis*). *Commun. Soil Sci. Pl. Anal.*, **47**: 1680-1690.
- DASGUPTA, D., GHATI, A., ABHIJIT, SARKAR, A., SENGUPTA, C. AND PAUL, G., 2015. Application of plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of *Sesbania bispinosa* on the growth of chickpea (*Cicer arietinum* L.). *Int. J. Curr. Microbiol. appl. Sci.*, **4**: 1033-1042.
- GOSWAMI, D., THAKKER, J. N. AND DHANDHUKIA, P. C., 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Fd. Agric.*, **2**: 1-19.
- HEGAZI, M. A., METWALY, M. M. S. AND BELAL, E.B., 2015. Influence of plant growth-promoting bacteria (pgpb) on coriander (*Coriandrum sativum*, L.) and dill (*Anethum graveolens*, L.) plants. *J. Pl. Product.*, **6**: 205-218.
- JHA, C. K. AND SARAF, M., 2015. Plant growth promoting rhizobacteria (PGPR): a review. *J. Agric. Res. Develop.*, **5**: 108-119.
- LOWRY, O. H., RESEBROUGH, N. J. AND FARR, A. L., 1951. Protein measurement with the folin-phenol reagent. *J. biol. Chem.*, **193**: 265-275.

- MAHADEVAN, A., 1984. In: *growth regulators, microorganisms and diseased plants*. Oxford and IBH Publishing Company, India., 31.
- MEENA, M. K., GUPTA, S. AND DATTA, S., 2016. Antifungal potential of PGPR, their growth promoting activity. *Int. J. Curr. Microbiol. appl. Sci.*, **5**: 235-243.
- MENG, Q., JIANG, H. AND HAO, J. J., 2016. Effects of *Bacillus velezensis* strain BAC03 in promoting plant growth. *Biol. Contr.*, **98**: 18–26.
- MANGMANG, J., DEAKER, R. AND ROGERS, G., 2014. Effects of plant growth promoting rhizobacteria on seed germination characteristics of tomato and lettuce. *J. Trop. Crop. Sci.*, **1**: 35-40.
- NOUMAVO, P. A., AGBODJATO, N. A., GACHOMO, E. W., SALAMI, H. A., MOUSSA, F. B., ADJANOHAOUN, A., KOTCHONI, S. O. AND MOUSSA, L. B., 2015. Metabolic and biofungicidal properties of maize rhizobacteria for growth promotion and plant disease resistance. *Afri. J. Biotechnol.*, **14**: 811-819.
- PAPENFUS, H. B., KULKARNI, M. G., STIRK, W. A., RENGASAMY, K. R. R., SALOMON, M. V., PICCOLI, P., BOTTINI, R. AND STADEN, J. V., 2015. Interactions between a plant growth-promoting rhizobacterium and smoke-derived compounds and their effect on okra growth. *J. Pl. Nutri. Soil Sci.*, (doi: 10.1002/jpln.201400556).
- PARK, Y. S., PARK, K., KLOEPPER, J. W. AND RYU, C. M., 2015. Plant growth-promoting rhizobacteria stimulate vegetative growth and asexual reproduction of *Kalanchoe daigremontiana*. *Pl. Pathol. J.*, **31**: 310–315.
- REGO, M. C., BORGES, F. I., FILIPPI, M. C. C. D., GONCALVES, L. A. AND SILVA, G. B. D., 2014. Morphoanatomical and biochemical changes in the roots of rice plants induced by plant growth-promoting microorganisms. *J. Bot.*, (doi: org/10.1155/2014/818797).
- ROUT, M. E., 2014. The plant microbiome. *Adv. Bot. Res.*, **69**: 279-309.
- SOUZA, R. D., AMBROSINI, A. AND PASSAGLIA, L. M. P., 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.*, **38**: 401-419.
- TANG, W. Y. AND BORNER, J., 1979. Enzymes involved in synthesis and breakdown of indoleacetic acid. In: *Modern methods of plant analysis*. (eds. K. Peach and M. V. Traceypp). Springer Verlag, Gohingen, Heidelberg: Vol. 7. pp. 238-241.
- TALLAPRAGADA, P., DIKSHIT, R. AND SESHAGIRI, S., 2015. Isolation and optimization of IAA producing *Burkholderia seminalis* and its effect on seedlings of tomato. *Songklanakarin J. Sci. Technol.*, **37**: 553-559.
- VAISHNAV, A., KUMARI, S. JAIN, S. VARMA, A.D.K. AND CHOUDHARY, D. K., 2015. Putative bacterial volatile-mediated growth in soybean (*Glycine max* L.) and expression of induced proteins under salt stress. *J. appl. Microbiol.*, **119**: 539-551.
- VEJAN, P., ABDULLAH, R., KHADIRAN, T., ISMAIL, S. AND BOYCE, A. N., 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-a review. *Mol. Div. Preserv. Int.*, **21**: 1-17.
- WU, Y. N., FENG, Y. L., PARE, P. W., CHEN, Y.L., XU, R., WU, S., WANG, S. M., ZHAO, Q., LI, H. R., WANG, Y. Q. AND ZHANG, J. L., 2015. Beneficial soil microbe promotes seed germination, plant growth and photosynthesis in herbal crop *Codonopsis pilosula*. *Crop Pasture Sci.*, **67**: 91-98.

Some Abstracts

PLENARY LECTURES

THE ADAPTIVE EVOLUTION OF CILIATED PROTOZOAN

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Protozoan is a big group of unicellular eukaryotes. They have very high biodiversity and distribute in various environments. So they are good objects for us to study the adaptive evolution. On one hand, we use the experimental evolutionary genomic method to study some fundamental biological questions in a model ciliate, *Tetrahymena thermophila*, such as the adaptive advantages of sexual and asexual reproduction; on the other hand, using the genomic techniques, we found some genetic resources involved in the adaptation of free-living/parasitic/mutualistic ciliates, and then construct the *Tetrahymena* cell lines to monitor/degrade/enrich the pollutants (e.g. heavy metals and DDT), and prevent the ciliates disease in fishery, and produce the zymine for ruminants combining molecular genetics tools and fermentation techniques

FUTURE TRENDS FOR SUSTAINABLE AQUACULTURE IN TURKEY

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Aquaculture is considered as a global industry to provide food for the world for many years. Cultured fish production was started at 1970s in Turkey. Aquaculture activities have been applied as intensive, semi-intensive and extensive culture types in earthen and concrete ponds, fiberglass tanks and in cages in commercial and family production type farms. It was a rainbow trout farm (*Oncorhynchus mykiss*) at first then followed by sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) after mid of 1980s and tuna took place in 2000s. Nowadays, additional to land based farms trout production can be seen in cages both in inland waters and sea waters. Aquaculture production was 79,031 tons in 2000 and it became 235,133 tons in 2014 and finally it reached 253,395 tons which was 43.04% of total fishery production of Turkey and 0.23% of the total aquaculture production of the world in 2016. It is stated that aquaculture production will increase 47.02 % in 2023 and become 500,000 tons with a 1,125,000,000 US dollar commercial value by the State Planning Organization of Turkey. With the improvement of aquaculture sector, aquaculture education in universities, many hatcheries and feed production companies as well as technological supplies such as nets and fiberglass tanks have been established in Turkey. It became an independent sector and represented by General Directorate of Fisheries and Aquaculture under Food Agriculture and Livestock, Ministry of Republic of Turkey. In Europe, Turkey ranks 6th in fish farming production and 3rd among Caucasian countries. Following plant production, animal husbandry and forestry, fisheries are considered one of four sub sectors (2.7%) of agricultural production with 0.3% in GNP (Gross National Product). Along with the development of aquaculture, some environmental problems were reported from water sources. Only one sustainable fish farm was reported so far. Therefore, it is aimed to overview about current status and future trends of Turkish aquaculture sector in this review.

HYPOVITAMINOSIS D AND CORONARY ARTERY DISEASE IN PAKISTAN

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Hypovitaminosis D is widespread in Pakistan with prevalence of vitamin D deficiency ranging from 70 - 92% among adults. **Objectives:** To find out any association of vitamin D deficiency with acute myocardial

infarction (AMI) in a Pakistani population and to investigate if vitamin D binding protein (VDBP) polymorphism has any relationship with this disease. It is a comparative cross-sectional study in which 246 patients (171 males and 75 females having an age between 20 to 70 years) with first AMI were recruited with informed consent from the National Institute of Cardiovascular Diseases, Karachi. Similarly, 345 healthy subjects (230 males and 115 females; age 20 – 70 years) were enrolled as controls with informed consent. Fasting serum samples of both AMI patients and controls were analyzed for 25 (OH) vitamin D, lipids and other related biomarkers using kit methods. DNA was analyzed for VDBP genotypes using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFL) based methods. Chi-square test and logistic regression were used for association of vitamin D deficiency and VDBP genotypes with AMI. **Results:** Mean serum/plasma level of 25(OH)D was 13.5 ± 14.0 ng/ml in AMI patients, which was significantly lower than the mean level 16.1 ± 11.7 ng/ml in healthy subjects ($p = 0.015$). Vitamin D deficiency [25 (OH)D serum levels < 20 ng/ml] was highly prevalent among AMI patients (83%) and healthy subjects (70.6%). When these proportions in two groups were compared, an association between vitamin D deficiency/insufficiency and AMI was found in this population ($p = 0.003$). Prevalence of vitamin D deficiency was high in all the major ethnic groups in this Pakistani population and mean serum levels of 25(OH)D among major ethnic groups were not found to be significantly different. An association between monthly household income and vitamin D deficiency was also found. Odds of having vitamin D deficiency was more than 3 times higher in the group with lower monthly household income (less than Rs. 50,000) compared to the group with higher (greater than Rs. 50,000) monthly household income [OR = 3.22; 95% CI (1.65-6.28)]. In subjects above 45 years, 1F-1F genotype of VDBP was found to be positively associated with the risk of AMI after adjusting for vitamin D levels, gender, BMI and LDL-cholesterol [OR = 9.86; 95% CI = 1.16 to 83.43]. **Conclusions:** Vitamin D deficiency/insufficiency is found to be associated with AMI in a population of Pakistani adults, and 1F-1F genotype of VDBP appears to increase the risk of AMI in subjects above 45 years of age.

DEVELOPMENT OF AQUACULTURE AND AQUARIUM SECTOR IN TURKEY

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The world population is estimated to be 10 billion in 2050. In this regard, one of the most important of the problems of the world is undoubtedly nourished. As an optimistic development in this regard; aquaculture appearance at a significant level compared to the agricultural sector in many countries. In archaeological investigations found some evidence of the aquaculture being made by the Egyptians for the first time in 2500 BC. Extensive marine farms of seabass, seabream, mullet and oyster were produced for the first time in Rome in the 6th century BC. Salmon is the first type of fish that is cultivated in cold waters. Modern aquaculture began about 40 years ago. Many Mediterranean countries took part in this development. Production of aquatic animals from aquaculture in 2014 amounted to 73.8 million tonnes. This total comprised 49.8 million tonnes of finfish, 16.1 million tonnes of molluscs, 6.9 million tonnes of crustaceans, 7.3 million tonnes of other aquatic animals including amphibians and 27.3 million tonnes of aquatic plants. Other major producers were China (60%), India, VietNam, Bangladesh and Egypt. Production of aquatic animals from aquaculture is 76.641.025 tonnes in world in 2015. In Turkey, aquaculture began in 1968 with the culture of inland water fish in the terrestrial environment. When examining the data year 2016 with respect to the date reached; our trout production is 99 712 tonnes and carp production has reached 196 tons. The production of marine fishes started in 1984. Aquaculture of marine fishes (151.794 ton in 2016) has entered into a rapid development process in the culture with sea bream (58.254 ton) and sea bass (80.847 ton) fishes, especially on the Aegean coast. All these economic fishes are valuing as a food source. The other fish sector is ornamental fishes and became very popular in all around the world. Most of the ornamental fish lovers usually keep them for recreational purpose or show their rich and high status as a symbol. The global import value for ornamental fish rose from US\$ 247.9 million in 2000 to US\$299 million in 2014. This sector is a multi-million dollar market with considerable growth in the last two decades. USA, Europe, and Japan are the largest markets for aquarium fish. Ornamental fishes come from Asia (65%) to this sector. Aquariums are kept in most shopping centers, cinema and conference halls in Turkey now. Unfortunately, in Turkey, there are not native fish species that are considered

ornamental fish species. Most of the ornamental fishes are exotic species and are imported from foreign country. The culture fish and the ornamental fish sector are the global components of international trade and development.

THE GOOD AND THE BAD OF P53

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The p53 protein is the most investigated and one of the most well-known proteins. p53 the gene that encodes p53 is evolutionary a very old gene. A p53-like gene was already present in early metazoan sponges and even in protists. From this early p53-like gene have three family members evolved that are called p53, p63 and p73 according to their molecular weight. These three proteins constitute the p53 family. p63 and p73 have overtaken functions in development while p53 has emerged to the most famous and most important tumor suppressor protein. p53 is mutated in almost every second tumor and tumors that retain wild type p53 have frequently mutations upstream or downstream of p53. In normal cells is the abundance of p53 low as its activity is not needed. However under conditions with an increased risk of tumorigenesis p53 levels rise and the protein becomes post-translationally modified resulting in its activation. Once p53 is activated it inhibits proliferation and induces cell death. While wild type p53 is a classical tumor suppressor protein it turns into an oncogene when it is mutated and enhances cell proliferation migration and metastasis of tumor cells. In my lab we are interested in the regulation and function of p53. To obtain a comprehensive view of the regulation of p53 we screened a library for novel regulators of p53. One of this novel regulators of p53 is Fam83F. In my presentation I will give a general overview over p53, present the screening of the library for novel regulators and discuss the regulation of p53 by Fam83F.

WHAT MADE ALANTOLACTONE A LEAD FOR ANTICANCER DRUG DEVELOPMENT?

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Sesquiterpene lactones are plant-derived bioactive compounds often used against inflammatory diseases in traditional medicines. In recent years, sesquiterpene lactones have gained a considerable attention for their potential anticancer activity due to the presence of α -methylene- γ -lactone moiety. Alantolactone, a sesquiterpene lactone component of *Inula helenium* has been extensively studied to characterize its molecular mechanism of action and potential chemotherapeutic applications in various cancer cell lines and animal models. Although, alantolactone has been shown to interfere with multiple cellular signaling molecules, induction of oxidative stress by disrupting cellular redox balance in cancer cells has been considered the major mode of action. As cancer cells contain higher level of ROS compared to normal cells, therefore, they are more vulnerable to oxidative stress. ROS-based anticancer drug development strategy holds the promise to set the cancer cells on the road to ruin as it can be applied more broadly against various human cancers of multiple origins irrespective of their genotype and are less likely to suffer from drug resistance. Preferential killing of cancer cells through induction of oxidative stress above a toxic threshold positions alantolactone as a lead compound for cancer therapy. In addition, alantolactone acts as a novel STAT3 inhibitor and potent chemosensitizer which made it a lead for overcoming drug resistance and improving efficacy of existing clinical drugs.

OUTSTANDING CHARACTERISTICS OF SOME CLASSIC ORTHOPTEROID SPECIES FROM PAKISTAN

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Orthoptera insects are major pests of agriculture and grazing lands throughout the world including Pakistan. Their members are mostly terrestrial, destructive to varieties of crops and vegetation. They are phytophagous, predaceous and omnivorous in nature. The nymph resemble with adult in structure features and habits. Orthopterans occur worldwide, except in the coldest areas. 25,300 species have so far been named in 40 families. 3 super-families i-e Tettigonoidea, Schizodactyloidea and Grylloidea belong to order ensifera and 05 Acridomorpha super-families vis: Acridoidea, Pyrgomorpha, Tetrigoidea, Eumastacoidea and Tridactyloidea of order caelifera are found in Pakistan. Beside this, some observation on Praying Mantids and stick insect was also made. The information given for each species includes its correct name and the more important once by which it has been known in the past together with a brief description and an indication of its size. As a further aid to identification, illustration representing the different taxonomic groups and life-forms are given. Details of distribution are provided, including a map for most species. In addition to this, life-cycle, ecology, behavior and most important of all, the damage caused by each species is presented. The economic importance of each species is presented on a nine-point scale covering the range from major pest of many crops to few records of minor damage. The Orthopteroid insects have a fearsome array of enemies ranged against them. The strain to endure in the face of such odds has given rise to a wide spectrum of adaptations. Different ways in which grasshopper, locust, crickets and their closely relatives have become adapted (for survival) to life it include: grovelling, camouflaged, (crypsis and the mimicry of leaves is more common in ensifera) self-burial, digging tunneling etc on or below the surface of the ground are documented. Further, food selection and feeding behavior in ensifera and caelifera along with identification tools of various taxon was also highlighted. Orthopteroid species has very peculiar and interesting reproductive behavior its mating duration was maximum i-e 70-86 hours in Hemiacridinae followed by 18-24 hours in Cyrtacanthacridinae and shortest i-e 13-40 minutes in Gomphocrinae and most wondering fact i-e eating of male during mating was reported in Mantinae (*Hierodula transcaucasica*) when female completely consumes its own male partner while mating. Present investigation will be of particular value to national plant protection and extension services in dealing with insects problems and hence make a direct and positive contribution to an increase in agricultural production which is so badly needed worldwide.

ROLE OF ADHESION PROTEINS IN METASTASIS

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Constitutive activation of mutant *K ras* (*Kirsten rat sarcoma viral oncogene homologue*) and distraction of E-cadherin-catenin complex (E-cadherin, α -catenin, β -catenin and γ -catenin) plays an important role in apoptosis, differentiation and cell proliferation. To compare the expression pattern of *K ras* and E-cadherin-catenin complex in normal and mutant colorectal cancer cell lines we addressed the expression analysis of *K ras* with reference to its association with adherent molecules in two colorectal cancer cell lines i.e. Caco-2 (wild type *K ras* served as a control) and DLD1 (heterozygous mutation at codon 13) at message level by qRT-PCR and translational level by western blotting. In DLD1 cell lines *K ras* showed slightly higher while α -catenin showed a slight lower (1.3 folds), β -catenin and E-cadherin showed significantly lower expression (4.2 fold decrease) as compared to Caco-2 cell lines. It can be inferred that a possible cross talk exists between *K ras* and adherent junction mediated signalling. Mutation at codon 13 (G to D) leads to the overexpression of *K ras* and reduced expression of adherent junction complex resulting in metastasis.

SECTION - I**CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY,
TOXICOLOGY**

- 1. BIOCHEMISTRY, BIOTECHNOLOGY AND BIOINFORMATICS**
- 2. CELL BIOLOGY, GENETICS**
- 3. HUMAN AND ANIMAL DISEASES**
- 4. MICROBIOLOGY**
- 5. MOLECULAR BIOLOGY**
- 6. PHYSIOLOGY**
- 7. TOXICOLOGY**

1. BIOCHEMISTRY, BIOTECHNOLOGY AND BIOINFORMATICS

PRELIMINARY STUDIES ON PROTEIN FRACTION BY POLYACRYLAMIDE GEL ELECTROPHORESIS AND BIOCHEMICAL COMPOSITION OF THE GENUS *THENUS* (CRUSTACEA: DECAPODA: SCYLLARIDAE), FOUND IN WATERS OF NORTHERN ARABIAN SEA

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Thenus is one of the most commercially significant fishery item of the seven *Scyllaridae* genera. The objective of the current work is to analyze the basic macro-nutrients present in shovel nosed lobster (*Scyllaridae*) and identification of species on protein level for further analysis as protein biomarkers. The estimated biochemical constituents: Protein (15.559 ± 0.776), Carbohydrate (12.73 ± 3.90), Lipid (5.743 ± 2.479) and Moisture contents (83.993 ± 2.570) of edible portion of *Thenus orientalis* revealed the nutritional quality of the species. SDS page analysis of protein resolved 4 loci, Molecular weight fraction showed muscles proteins ranged from 282.26 Daltons to 637.32 Daltons. According to size, *Thenus orientalis* possess significant linear correlation between carapace vs total length and total length vs total weight (0.93 and 0.74) respectively. The five enzyme system; Catalase (CAT), Carbonic Dehydrogenase (CA), Creatinine Kinase (CK), Amylase (AMY) and Peroxidase (PRX) selected for the allozyme analysis. The number of loci and their relative mobilities for each allele was observed and results have shown the significant differences in the similarity matrix for the loci where fixed differences were observed.

VARIATIONS IN RENAL AND HEPATIC PROFILE OF HYPOTHYROID SUBJECTS IN LAHORE, PAKISTAN

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About 6.6% of world population is influenced with thyroid malfunctions with high proportion of females. Liver has an ultimate role in metabolizing thyroid hormones and also modulates their systemic effects. Concurrently, thyroid hormones regulate renal hemodynamics and basal metabolic rate of most cells. Therefore, both are vital for each other to maintain the integrity and homeostasis of the body. To evaluate the effect of hypothyroidism on liver and renal parameters and effectiveness of the thyroid treatment (thyroxin) on these factors in hypothyroid subjects. A total of 110 subjects (20 subclinical and 40 overt hypothyroid) between the ages of 15-50 years from different hospitals and 50 healthy euthyroid controls of similar age group from local population were recruited in the investigation. Moreover, follow up study of nearly twenty registered patients was also carried out after taking thyroxin for three months. Serum level of fT_4 , fT_3 and TSH were analyzed through Radioimmunoassay (RIA) whereas levels of AST, ALT and creatinine were assessed in the serum of the participants using chemistry analyzer. Serum level of ALT and AST were found to be significantly increased ($P \leq 0.05$) in overt hypothyroid patients compared to controls and after treatment the concentration of both enzymes was decreased non significantly in follow up group. Subclinical group also exhibited non-significant elevation in ALT and AST level. Creatinine level was also significantly ($P \leq 0.05$) increased in both subclinical and overt group and found non-significantly decreased in follow up group compared to overt group after taking thyroxin. This study concludes that a positive correlation of ALT, AST and creatinine with TSH level and negative correlation with serum thyroid hormones (fT_3 and fT_4) is observed in hypothyroid group that is reversible after thyroid replacement therapy. This study was funded by Higher Education Commission, Pakistan.

CHARACTERIZATION OF ALPHA AMYLASE FROM BACILLUS SUBTILIS FROM ISOLATED FROM LOCAL ENVIRONMENT

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The conditions prevailing in the industrial applications in which enzymes are used are rather extreme, especially with respect to temperature and pH. Consequently, there is a continuing demand to increase the stability of enzymes and to meet the necessities set by specific applications. In this respect, thermostable enzymes have been suggested to be industrially relevant. In this analysis, α -amylase, a well-established representative of thermostable enzymes. This work was accompanied with the aim of isolating α -amylase producing *Bacillus subtilis* from local soil samples. Fifty bacterial strains were isolated from 28 soil samples collected from food industries dumping sites. On the basis of the morphological and biochemical characterization, five bacterial strains were screened. The maximum production of extracellular amylase by *Bacillus spp* was optimized in a submerged fermentation. The maximum enzyme production was obtained on M1 medium comprising molasses, dextrin, sucrose and Trypton. The production of the enzyme was maximum at 10 h after inoculation. The effect of incubation period, pH of the medium and incubation temperature was optimized. The maximum production of enzyme were attained at 35°C and pH 7. The maximum enzyme production was obtained on M1 medium comprising molasses, dextrin, sucrose and Trypton. These strains showed maximum α -amylase production at following optimum conditions: 18 h inoculum age; 37°C temperature; 48 h incubation time; 2% inoculum size; GCU-DAB-09A at pH 6, GCU-DAB-12E(a), GCU-DAB-02A and GCU-DAB-22A at pH 8, and GCU-DAB-23A at pH 9; GCU-DAB-02A, GCU-DAB-09A(b) and GCU-DAB-12E. The enzyme produced from all strains was originate to be stable at 1% starch concentration and pH 7. The enzyme produced from GCU-DAB-02A, GCU-DAB-12E (a) and GCU-DAB-23A was stable at 45°C, though the other two i.e., GCU-DAB-09A (b) and GCU-DAB- 22A alpha-amylase exhibited maximum stability at 40°C. These bacterial isolates possesses the high ability to degrade starch into its basic constituents, may find potential biotechnological applications in industry.

CORRELATION BETWEEN CIRCULATING BIOMARKERS OF OXIDATIVE STRESS AND TUMOR LYSIS SYNDROME (TLS) FROM LAHORE-PAKISTAN

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Tumor lysis syndrome become cause of metabolic disorder occur instinctively after anticancer treatment. It occurs when cytotoxic therapy that is radio or chemotherapy damaged cancerous cells particularly leukemia or lymphoma's cells. TLS is a serious disorder become cause of hyperuricemia, hyperkalemia, hyperphosphatemia, hypocalcaemia which further leads to fatal complications such as kidney, heart, musculoskeletal and brain disturbance. 5.0 ml venous blood sample of 70 patients of TLS and 70 Blood sample of healthy individuals was taken in clotted gel vial from oncology department, Mayo Hospital and Jinnah Hospital Lahore. Blood was further processed for the estimation of Reduce Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Nitric oxide (NO), Vitamin A, Vitamin C and Vitamin E, Neuraminidase and Electrolytes concentration by flame photometer (Na^+ and K^+) and interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF- α) by kit method. MDA level in HCV patients was increases remarkably (13.33 ± 1.44) and in control persons (3.17 ± 0.61) and it was statistically significant ($0.000 < 0.05$). The serum sodium (Na^+) level also elevated in TLS patients (169.21 ± 7.43) as compared to the control persons (124.31 ± 6.19) and statistically significant ($0.001 < 0.05$). Serum cytokine level TNF-alpha and IL-2 also raised in TLS patients (54.44 ± 3.41) and (223.37 ± 4.29) respectively. Nitric oxide level in TLS patients recorded as (34.22 ± 2.21) and in control (21.65 ± 3.61). Biochemical study of the TLS showed that oxidative stress, Cytokines and electrolyte balance play a key role in the development of Tumor lysis syndrome. It is concluded

that the patients suffering from TLS has remarkably high lipid peroxidation due to which the level of MDA was increases remarkably while the level of SOD, Catalase and GSH was decreases. While the cytokines level in TLS patients also elevated remarkably.

DETECTION AND VALIDATION STUDIES OF TRACE METALS, PROTEIN AND STEROID IN DIFFERENT ORGANS OF LOCAL AND BRAND MEAT (POULTRY, CATTLE AND FISH)

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Present study was conducted to investigate the concentration of trace metals (Na, K, Fe, Zn, Cu, Ni, Mg, Co, Cd and Pb) in different organs (heart, liver, muscles, wings, neck) of poultry (domestic, broiler), cattle (mutton, beef) and fish available from local and branded markets of Lahore. Atomic Absorption Spectroscopy and Flame Photometry were used to estimate the contamination of these metals in meat. The concentration of trace metals in different organs showed great variation. The estimated values of Na, K, Fe, Mg and Ni were under the tolerable levels cited by international standards; World Health Organization (WHO) and Australia New Zealand Food Authority (ANZFA). The Co, Cd and Pd were absent in meat samples but broiler, menu and zenith wing samples had high concentration of Zn and Cu exceeding the permissible limits. Rapid and sensitive Lowry method was adopted for scrutinizing the protein content in internal organs (heart, liver, breast, wings, neck and leg) of poultry meat (domestic, broiler, zenith and menu). The result showed that liver of domestic chicken is a rich source of protein. Quantitative evaluation of steroid (testosterone) in chicken through High Performance Liquid Chromatography confirmed that the level of testosterone in different organs didn't exceed the acceptable limit.

POPULATION STUDIES USING STR LOCI IN HUZA COMMUNITY

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In Forensic casework DNA plays a vital role using basic techniques of Biotechnology. On account of achievements accomplished in past three decades, forensic DNA analysis has become a key to convict and exonerate the suspects and to identify the victims of criminal cases, accidents and mass disasters. A lot of Markers has been tested and applied in Forensic investigations to exploit justice in criminal cases and solve the paternity related issues. However there has been observed some existing imperfections and limitations in the use of conventional STR system as in conditions of highly degraded DNA. In the current study optimization and population data of 10 autosomal loci multiplex system was accomplished comprising on 10 autosomal STRs including SE33, Penta E, Penta D, CSF1PO, D7S820, TPOX, D18S51, D13S317 and FGA has been presented. The combined power of Discrimination of 10 markers in Hunza population was calculated as 0.9999999999999457. Combined Matching Probability was $5.33304826319092 \times 10^{-13}$. Combined Power of Exclusion was 0.99999937114115.6. More over the multiplex system was validated by following SWGDAM (Scientific Working Group on DNA Analysis Methods) guidelines concerning stability, sensitivity, species specificity, accuracy and precision, DNA mixtures, population distribution genetics, forensic and paternity statistical parameters, low copy number of DNA and studies based on PCR amplification.

CHARACTERIZATION OF THERMOSTABLE AMYLASE FROM *BACILLUS* SPECIES AND ITS POTENTIAL APPLICATION IN FOOD INDUSTRY

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Amylase is an extracellular microbial enzyme known for its wide industrial applications. In this study, *Bacillus* species was found to be express it at 4th hour till the cell go to dead phase in growth medium supplemented with 1% starch. The enzyme exhibited optimum activity at 70°C, 1% starch, pH 7, 0.03 % triton X-100, 0.03 % tween 20, 0.1% surf excel, 1% banana peel, 1% casein and 1% starch. Increase in starch concentration decreases its expression. Its activity was improved in the presence of Fe and Mg ions but NH₄ ions and EDTA decreased its activity. The enzyme was isolated and purified using gel filtration chromatography and DEAE chromatography. Its potential application in baking industry was checked but it needed to be improved before this isolate can be commercialized.

IN HOUSE METHOD VALIDATION ON COMPLEMENT ACTIVATION USING COAGULATION SYSTEM

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The complement and coagulation systems are evolutionarily ancient, phylogenetically related and important ingredients of innate immune effectors which sense incoming signals of danger. Possible interactions between both systems and the precise pathways involved, by which activated coagulation factors may trigger the complement cascade, still remain unclear. Therefore, the key coagulation factors (F) were investigated for their potency to activate the complement system and to invitro generate C3a or C5a. Strong interactions of both cascades were found as determined by a dose- and time-dependent cleavage of the key players of the complement system, C3 and C5, by FXIa, FXa, FIXa, thrombin and plasmin. The cleavage products, C3a and C5a, exhibited a chemotactic activity for either human mast cell line (HMC-1 cells) or neutrophils respectively. The protein sequence analysis of the generated C3a and C5a confirmed that their sequences are similar to those of the naturally existing anaphylatoxins C3a and C5a, suggesting that the named clotting proteases may mimic the natural pattern of C3 and C5 cleavage. The FXIa-, FXa-, FIXa-, thrombin- and plasmin-induced C3 and C5 cleavage activity could be blocked by the serine protease inhibitor leupeptine and by the sulfonyl fluoride type inhibitor pefabloc. Furthermore, Michaelis-Menten analyses revealed complement cleavage efficacy of all measured coagulation factors follows a Km-order of FXa<FXIa<plasmin<thrombin<FIXa<control. Present data provide first evidence of C3-cleavage by various clotting serine proteases and indicate a strong relation between the coagulation and complement cascades.

EFFECT OF DIETARY VITAMIN E SUPPLEMENTATION ON CATALASE ACTIVITY OF GRASS CARP (*CTENOPHARYNGODON IDELLA*)

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Present work was conducted to investigate the effect of dietary vitamin E (VE) supplementation on catalase activity in grass carp (*Ctenopharyngodon idella*). The fish were fed with four types of diet containing the different quantity of vitamin E viz. T₁ (Control) VE-0 mg/Kg, T₂ VE-200 mg/Kg, T₃ VE-350 mg/Kg and T₄ VE-500 mg/Kg. The fish were fed three times in a day for one month. After one month fish was dissected and organs viz. muscle, gills and liver were separated to evaluate the catalase activity. The catalase activity was decreased in all selected organs of grass carp fed with diet T₄ (VE-500 mg/Kg) as compared to control T₁ having no vitamin E after 30-day. It was observed that the fish fed with T₁ diet showed highest catalase activity in liver, gills and muscles as 20.34±0.99, 14.68±0.90 and 12.76±0.87 U mL⁻¹, respectively. In other groups the activity decreased with the following order: T₂>T₃>T₄. The highest CAT activity was observed in liver followed by gills and muscles.

ARTIAL PURIFICATION AND KINETIC CHARACTERIZATION OF RENAL CATALASE FROM CARNIVORE FISH, *CHANNA STRIATA* EXPOSED TO PESTICIDES MIXTURE, DELTAMETHRIN+ENDOSULFAN

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The current research work was conducted to purify the renal catalase (CAT) activity of carnivorous fish, *Channa striata* exposed to the sub-lethal concentration (1/3rd of LC₅₀) of deltamethrin (DM)+endosulfan (END) mixture for 14 days. Catalase was partially purified by using ammonium sulphate precipitation technique. Spectrophotometric method was used to analyze the enzyme activity. The lowest CAT activity and specific activity was noted from the crude extract of DM+END exposed *C. striata* renal tissue as compared to control. As a result of partial purification of renal CAT enzyme lowest specific activity was observed in exposed fish (80.94±0.00 U mg⁻¹) in relation to control (88.97±1.41 U mg⁻¹). The fold purification of renal CAT from exposed and control fish was calculated as 1.24±0.00 and 1.28±0.01, respectively. The percentage recovery was about 58.07±1.41 and 63.23±1.41 % for exposed and control fish, respectively. Results of kinetic characterization showed that the maximum renal CAT activity was observed at 6.5 and 30°C respectively.

ASSESSMENT OF ANTIBACTERIAL, BIOFILM INHIBITION AND CELL PROLIFERATION EFFECT OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING *MORUS NIGRA* EXTRACT

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The development of resistance among contagious pathogens is the major concern in the whole world and

there is a serious need to combat the pathogens. The current study was aimed to synthesize the silver nanoparticles using *Morus nigra*, characterize and investigate their antibacterial activities against human-associated bacterial pathogens viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* through agar well diffusion method, tetrazolium salt assay, and crystal violet assay. Characterization of green synthesized nanoparticles was done through UV-viz spectrophotometry, Scanning Electron Microscope, and Fourier-transform infrared spectroscopy. In agar well method results indicated that maximum inhibition of *Escherichia coli*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* with 12.33 ± 0.57 mm, 12.00 ± 1.73 mm, 13.3 ± 12.08 mm, and 13.00 ± 0.00 mm zone of inhibition while *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Serratia marcescens* showed moderate inhibition with 7.00 ± 1.00 mm, 8.00 ± 1.00 mm, 8.66 ± 0.57 mm zone of inhibition. Cell viability assay, antibiofilm assay, supported the results of agar well method. It was observed that extracts of *Morus nigra* indicated the significant inhibition of all tested clinical bacterial pathogens. Therefore, results of current study were more effective and could be a potential source of new antimicrobial drug from green source.

POTENTIAL OF AGRICULTURAL WASTE FOR THE PRODUCTION OF BIOPLASTIC BY SOLID STATE FERMENTATION

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Polyhydroxybutyrate (PHB), a bioplastic, has attracted the attention in the recent years due to its ability to replace the polyethylene, a non-biodegradable plastic. The aim of the present research work was to evaluate the potential of different agricultural wastes like wheat bran, rice polishings and corn cob for the synthesis of PHB by *Bacillus thuringiensis* FCBP-SB-0002 using solid state fermentation. Optimization of different components of basal media and various physical parameters was also performed. Maximum PHB yield (420 mg/100g) was achieved on fermentation of rice polishing at substrate water ratio of 10:36 at 72 hours of incubation time, pH 7, temperature of 30°C by addition of 1 mL inoculum. Addition of different optimum levels of ionic salts (1.5% of $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 2% MgSO_4 and 2% NaCl) and nitrogen sources (0.75% urea and 1% corn steep liquor) increased the PHB production to 680 mg/100g respectively. Identification of PHB was done by FTIR analysis and was found to be 98% pure in comparison to standard by spectrophotometric method. The outcomes of the present study indicated that agricultural wastes can be used for the cheap production of bioplastic. This strategy will also help to reduce environmental pollution caused due to disposal of this waste material.

ASSESSMENT OF WATER QUALITY PARAMETERS OF KALAR KAHAR LAKE, PAKISTAN.

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The present study was designed to demonstrate the physico-chemical parameters of water of Kalar Kahar Lake from October 2015 to September 2016. Water samples were collected throughout the years on monthly basis for the measurement of water colour, Taste, pH, electrical conductivity, Total dissolve solids, Calcium, Magnesium, Chloride, total hardness and total alkalinity. The water of the lake was greenish in colour and had salty taste. The mean values of chemical parameters were observed such as pH (9.3 ± 0.42), Electrical Conductivity (2351.7 ± 252.5), Total dissolve solids (1840.2 ± 273.2), Calcium (278 ± 72.8), Magnesium (282 ± 95.8), Chloride (605 ± 38.18), Total Hardness (965 ± 131.4) and total alkalinity (764 ± 56.6). The parameters were compared with the water quality standard to estimate the water quality. The value for the Total dissolve solids, Hardness, alkalinity and chloride were above the values of W.H.O maximum permissible values.

BIOSORPTION OF CD (II) FROM AQUEOUS SOLUTION BY CHITIN EXTRACTED FROM PORTUNID CRAB (*CHARYBDIS FERIATA*): ADSORPTION BEHAVIOR AND MECHANISM ASSESSMENT

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Heavy metal contamination has become a serious problem all around the world as attributed to its great damage to the environment and public health. Among them, cadmium (Cd) considered as one of the most toxic metal which create acute and chronic toxicities even at low concentration. In the previous years, the contamination of Cd increased as the results of industrial influences, therefore detoxification of Cd is a necessity for the eco-friendly existence of industries. The aim of this study was to evaluate the adsorption performance and mechanism of second most abundant biomaterial (chitin) extracted from the Portunid crab (*Charybdis feriata*) for removal of Cd (II) in aqueous solution. The effects of operating parameters, viz., contact time, initial concentration and pH on Cd removal efficiency were studied. The data for adsorption process satisfactorily fitted to the Langmuir model than Freundlich model. The interactions between crab chitin and Cd (II) ions were investigated by qualitative analysis methods (FTIR and SEM-EDS). The crab chitin showed the great adsorption capacity for Cd (II) in a wide ranging environment (1 to 100 ppm) by removal efficiency 80–99.9% and designates the capacity of crab chitin to sequester Cd (II) in industrial effluents. In conclusion, the abundant and cheap seafood bio-waste, crab shell can be an effective candidate for removal of Cd from aqueous solutions and could potentially contribute to toxic pollutant removal from the released wastewater.

RESISTANCE AND UPTAKE OF CADMIUM BY YEAST, *PICHIA HAMPSHIRENSIS* 4AER, ISOLATED FROM INDUSTRIAL EFFLUENT AND ITS POTENTIAL USE IN DECONTAMINATION OF WASTEWATER

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Pichia hampshirensis 4Aer is first ever used yeast for the bioremediation of environmental cadmium (Cd⁺²) which could maximally remove 22 mM/g and 28 mM/g Cd⁺² from aqueous medium at lab and large scales, respectively. The biosorption was found to be the function of temperature, pH of solution, initial Cd⁺² concentration and biomass dosage. Competitive biosorption was investigated in binary and multi-metal system which indicated the decrease in Cd⁺² biosorption with increasing the competitive metal ions attributed to their higher electronegativity and larger radius. FTIR analysis revealed the active participation of amide and carbonyl moieties in Cd⁺² adsorption confirmed by EDX analysis. Electron micrographs summoned further surface adsorption and increased cell size due to intracellular Cd⁺² accumulation. Cd⁺² was the causative agent of some metal binding proteins as well as an prodigious increase in glutathione and other non-protein thiols levels which is the crucial for the yeast to thrive oxidative stress generated by Cd⁺². Our experimental data was consistent with Langmuir as well as Freundlich isotherm models. Thermodynamic and kinetic studies revealed the Cd⁺² biosorption as exothermic, spontaneous and feasible process. The yeast obeyed pseudo second order kinetic model which make it an effective biosorbent for Cd⁺². High bioremediation potential and spontaneity and feasibility of the process make *P. hampshirensis* 4Aer an impending foundation for green chemistry to exterminate environmental Cd⁺².

MULTIPLE HEAVY METAL RESISTANT *STAPHYLOCOCCUS SCIURI* (A-HS1) ISOLATED FROM INDUSTRIAL EFFLUENTS: ITS POTENTIAL USE IN CR⁺⁶ REDUCTION

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A chromium-resistant bacterium was isolated from industrial wastewater effluent and identified as *Staphylococcus sciuri* (A-HS1) on the basis of morphological, biochemical tests and 16S rRNA ribotyping results. *S. sciuri* (A-HS1) demonstrated optimum growth at 37 °C and pH 7. *S. sciuri* (A-HS1) was able to resist Cr⁺⁶ (25 mM) as well as other heavy metals such as As⁺² (19 mM), Pb⁺² (18.5 mM), Zn⁺² (17 mM), Cu⁺² (2.5mM), Cd⁺² (3 mM), and Ni⁺² (1.5 mM). Biochemical characterization of chromate reductase enzyme showed its optimal pH as 8.0 and optimal temperature as 40°C. Chromate reductase enzyme activity was stimulated only by Mg⁺² among other metals tested. Chromium biosorption efficiency (q) of *S. sciuri* A-HS1 was 42, 73, 85 and 31 mM/g after 2, 4, 6 and 8 days, respectively. Hexavalent chromium presence did not stimulate activities of APOX, SOD and CAT in significant quantities however a decrease in activities of these antioxidants were observed i.e., APOX (11%), SOD (8%), and CAT (3%), respective to the normal growth conditions. An increase in glutathione and other non-protein thiols levels played a significant role in combating the oxidative stress generated by the toxic metal cations. Pilot study demonstrated that *S. sciuri* A-HS1 was able to remove 87% Cr⁺⁶ from tannery effluent and 97 % Cr⁺⁶ from industrial effluent within 6 days of incubation. The present study revealed that *S. sciuri* A-HS1 may act as a potential candidate for the bioremediation of hexavalent chromium contaminated environmental sites.

POTENTIAL USE OF BACTERIA ISOLATED FROM INDUSTRIAL EFFLUENTS IN DECONTAMINATION OF WASTEWATER CONTAINING AZO DYES

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Pakistan has a broad network of industries which is greatly required for the economic development of the country. Synthetic dyes, especially azo dyes are of great use in many industries such as textile, cosmetics, paper, leather and pharmaceutical etc. The discharge of wastewater from these industries proved to be fatal for the ecosystem as well as living organisms. As dyes are non- degradable so they remain stable in the wastewater. Physiochemical methods use to degrade pollutants are not economic friendly and they generate by products which are also toxic in nature. A recent approach is to treat the wastewater with microorganisms which reduces the cost of wastewater treatment. The present study was designed to isolate dye degrading bacteria and to characterize them morphologically and biochemically. Wastewater samples were collected from Kot Lakhpat industrial estate, Lahore and azo dye resistant aerobic and anaerobic bacteria were isolated. The dye degradation conditions were optimized and degradation was also checked on large scale. Microbial and phytotoxicity of this treated wastewater was also checked. TLC, HPLC and FTIR were also performed to analyze the degraded products.

RESISTANCE AND RESPONSE OF YEASTS AGAINST TOXIC POLLUTANTS: A STRATEGY TO PURIFY INDUSTRIAL WASTEWATER

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This study examines the bioremediation potential and cadmium-induced cellular response on a molecular

level in *Candida tropicalis* 3Aer. Spectroscopic analysis clearly illustrated the involvement of yeast cell wall components in biosorption. Cadmium bioaccumulation was confirmed by TEM, SEM, and EDX examination. TEM images revealed extracellular as well as cytoplasmic and vacuolar cadmium nanoparticle formation, further validated by presence of *ycf1* gene and increased biosynthesis of GSH under cadmium stress. Fourteen proteins exhibited differential expression and during cellular redox homeostasis are found to involve in nitrogen metabolism, nucleotide biosynthesis, and carbohydrate catabolism. Interestingly, *C. tropicalis* 3Aer is equipped with nitrile hydratase enzyme, rarely been reported in yeast. It has the potential to remove nitriles from the environment. The Cd⁺² toxicity not only caused growth stasis but also upregulated the cysteine biosynthesis, protein folding and cytoplasmic detoxification response elements. Yeast was also able to degrade azo dye present in the environment. This study suggests that yeasts can be used as potential candidates for bioremediating environmental pollution.

SUBMERGED CULTIVATION OF *SACCHAROMYCES CEREVISIAE* AS SINGLE CELL PROTEIN.

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The proteins obtained from microorganisms are known as single cell proteins. In the present study, three types of cereal grain extracts were taken i.e. wheat, maize and barley. The extracts of each cereal grain were used in submerged fermentation by *Saccharomyces cerevisiae*. In the fermentation yeast was allowed to grow in aqueous medium containing nutrients in dissolved condition. The proximate analysis of cereal grain extract was performed prior to use for the growth of yeast. The cereal grain extracts constituted sufficient of nutrients essential for the growth of yeast in submerged fermentation process. The results showed increase in nutrient composition of fermented cereal grain extracts. There was significant increase in the protein content of the fermented cereal grain extracts. The protein content of non-fermented wheat was found to be 21 % and the increased value of protein content in fermented wheat was estimated as 24 %. Similarly the protein content in non-fermented maize and barley was measured as 17% and 15% respectively which showed increase in values in fermented maize and barley i.e. 22% and 19% respectively. The increase in the crude fiber content was observed also in the fermented grain extracts. The fiber content of fermented wheat, maize and barley was increased to 2.1 % in wheat, 2.3 % in maize and 2.4 % in barley. Increase in enzyme activity of amylase, protease and phytase was also observed in fermented grains as compared to non-fermented grain extracts.

PURIFICATION AND CHARACTERIZATION OF CLONED β -XYLOSIDASE FROM *BACILLUS LICHENIFORMIS* ATCC 14580 INTO *E. COLI* BL21 FOR SACCHARIFICATION OF PLANT BIOMASS

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Purification and characterization of cloned β -xylosidase enzyme from *Bacillus licheniformis* ATCC 14580 in to *Escherichia coli* strain BL21 using pET- 21a as an expression vector was carried out in this research work. Recombinant β -xylosidase enzyme was purified by ammonium sulphate precipitation, followed by single step Immobilized Metal Ion affinity Chromatography with 2.58 fold purification having 20.78 Umg⁻¹ specific activity and recovery was 33.75%. Molecular weight of the purified β -xylosidase, 52 kDa, was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The enzyme was stable upto 90°C with a broad pH range of 3 - 8 pH units with optimum temperature 55°C and pH 7.0. The enzyme activity was increased in the presence of metal ions especially Mg⁺², and did not considerably affected in the presence of EDTA. However, an addition of 1% Tween 80, β -mercaptoethanol and DTT resulted in increase of enzyme activity by 6%, 18% and 22%, respectively. Organic solvents with a concentration of 10 - 40% did not showed any effect upon enzyme activity. The β -xylosidase enzyme possesses the ability of bioconversion of plant

biomasses like wheat straw, rice straw and sugarcane bagass and can be used in the bioconversion of natural biomasses into simple sugars which could be further used for the production of biofuel.

HYDROGEL CONTAINING AMNION PROMOTE DEEP SECOND DEGREE BURN HEALING IN RAT MODELS

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Burns are the serious traumatic event that can cause by various means like through scalding, from radiation and chemicals such as strong acids. World Health Organization (WHO) documented each year, about 300,000 deaths occurred worldwide. The majority are the children and the elderly people. Burns could be classified into three categories; First-degree burn, second-degree burn and the third- degree burn. This study include healing effect of hydrogel on rat burn model. The albino rats were used to create the burn model and subjected to second-degree partial thickness burn by exposing their shaved skin with 95°C for 8 seconds. After the three-day interval of post-wounding, the synthetic hydrogel applied. The hydrogel application soothes the wound irritation. Similarly, the dried amnion also applied to the wounded area along with gel to maximize the wound recovery and tissue integrity. The procedure continued until 14 days and the wound healed up. The skin of control rats were compared with the skin of rats on which hydrogel with amnion was applied. Results showed that the skin in experimental rats not only healed in fewer days as compared to control but also it showed close resemblance to the texture of normal skin. The future prospect of current research work encompasses the delicacies of burn victims. As rats had been considered as the most versatile animal for research purpose therefore being the trial-based method, it has been concluded that effect burns of second-degree could be minimized with this new experimentation.

BIOINFORMATICS BASED CHARACTERIZATION OF HYPOTHETICAL PROTEINS AND ITS APPLICATION IN DISEASE TREATMENT

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Completely sequenced organisms have some uncharacterized proteins know as hypothetical proteins, which are the gene products merely predicted by in-silico approaches and have no experimental evidence. Structural and functional characterization of hypothetical protein reveals crucial aspects of microorganisms especially pathogens of human. Here, we discuss recent methods in hypothetical protein characterization and biomedical applications including drug and vaccine development using these proteins. Structural characterization of proteins provides hints to predict their functions. Functionally annotated hypothetical proteins with their completely known structures, can act as novel drug targets for disease treatment, due to their involvement in pathways related to disease causing organisms. These proteins are also being used in vaccine production and sero-diagnosis. In current era of drug and antibiotic resistance these proteins can be novel targets to treat the diseases. Identification and characterization of most of hypothetical proteins is under observation and will be most promising genomic and bioinformatic techniques in structure based drug designing and vaccine production in future.

CHARACTERIZATION OF PROTEOLYTIC ACTIVITY OF *BACILLUS SUBTILIS* ISOLATED FROM TANNERY EFFLUENT

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The current study was aimed to screen and isolate protease producing bacteria from soil sample collected

from tannery effluent of Kasure District, Punjab. Soil sample was serially diluted followed by spreading on LB-Agar plates and incubation at 37°C for 48 hours. Total 15 bacterial colonies showed clear zones of inhibition. Isolate no.6 showed maximum 30mm zone of inhibition and it was identified as *Bacillus subtilis* by 16S rRNA. Various physiological characteristics such as pH, temperature, fermentation time, and effect of additives, inhibitors, oxidants, surfactants and organic solvents were studied. Proteolytic activity of *B.subtilis* was also evaluated quantitatively. It showed maximum activity at pH 5.05, 6.36 and 4.07 and 50°C. The presence of additives like Fe, Hg, Ba, Cu and Al increase the proteolytic activity. The protease showed maximum activity on surfactants (Cetyl Trimethyl, Ammonium Bromide, Sodium Dodecyl Sulphate and TWEEN-20), inhibitors (Ethylene Diamine Tetra Acetic acid) and oxidants (Hydrogen peroxide). The protease showed maximum activity on organic solvents such as Acetone, Ethanol, Iso-propyl alcohol, Diethyl ether, n-hexane and Chloroform. The protease also showed its use as detergent.

SCENEDESMUS DIMORPHUS NT8C CONVERSION OF SUGARCANE BAGASSE INTO BIODIESEL

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Continuously increasing both human population and their energy demand has resulted into the dangerous outcomes of deforestation and fossil fuels' exploitation. Consequently, we are left with the only choice to extract different fuels such as biodiesel, bioethanol, biohydrogen and biomethane from non-food biomass and industrial effluents which otherwise represent a major portion for solid waste management. In the present study *Scenedesmus dimorphus* NT8c was cultivated photoautotrophically and mixotrophically on sugarcane bagasse hydrolysate (SCBH). Green microalga was analyzed by studying its biochemical composition and growth parameters in both autotrophic and mixotrophic cultivations. Lipids were further analyzed through GCMS. *Scenedesmus dimorphus* NT8c cultures fed with sugarcane bagasse were found to have high growth rate, high biomass productivity, high proteins and more lipids as compared to the photoautotrophic cultivation. Microalgae grown mixotrophically are capable of photosynthesizing while metabolizing and assimilating organic carbon. In addition, consumption of agri-industrial food wastes help to resolve issues of their dumping and pollution of natural environments.

COMPARISON OF BIODEGRADATION POTENTIAL OF SELECTED DISPERSE TEXTILE DYES BY TWO INDIGENOUS BROWN ROT FUNGI AND STUDY OF LIGNOLYTIC ENZYMES

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Current study was designed to investigate the ability of *Daedalea dickinsii* IEBL-02 and *Piptoporus betulinus* IEBL-03 to decolorized disperse textile dyes. Biodegradation of disperse violet S3RL, disperse orange S2RFL and disperse red W4BS was monitored along with secretion of lignolytic enzymes. The decolorization process was observed for 10 consecutive days with the analysis of process on each day. The results showed that *Daedalea dickinsii* IEBL-02 (70-80 %) has the more potential of biodegradation of disperse dyes while *Piptoporus betulinus* IEBL-03 (47-59 %) has the least. The biodegradation process of dyes was optimized by

Response Surface Methodology with *D. Dickinson* IEBL-2 and more than 90 % biodegradation was achieved. The study of lignolytic enzymes i.e. lignin peroxidase, manganese peroxidase and laccase showed that *D. dickinsonii* IEBL-02 produced most active enzymes. Higher enzymatic activities related with more degradation indicated that these are involved in decolorization process. Enzymes showed maximum activities at 30 °C and pH 6.5 with good affinity towards their substrates as indicated by kinetic parameters. This study will bring the attention of other scientist to use brown rot fungi for the treatment of wastewater.

POTENTIAL OF BLACK SOLDIER FLY (DIPTERA: STRATIOMYIDAE) IN COMPOST MAKING

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With the increasing human population, production of waste has been increased many fold, due to which its disposal has been a major civic issue to handle. Black soldier flies (BSF) (*Hermetia illucens*), stratiomyidae dipterans which feed on dead material of plant and animal origin, and make the same into compost. Waste originating from plant and animal remains, constitute a major portion of municipal waste. If ability of BSF is used in decomposing of community wastes, human settlements can be cleaned on one hand, and the end product (compost) can be utilized for enrichment of soils to crop production. Considering above mentioned facts in view, the current study was planned, to evaluate the potential of BSF on various waste materials for compost making. For the purpose, the commonly available waste materials viz., kitchen waste, poultry liver, cow dung and vegetable & fruit peels were collected from Multan. Each treatment was replicated four times and data was analyzed statistically by using SPSS with 5% level of significance. It was observed that BSF larvae were most efficient to decompose kitchen waste as compared to other materials and convert it more rapidly into compost. It was also observed that the flies performed more efficiently in environments having Relative Humidity ranging 60 to 70 %, as compared to less than that. No fungal effects were observed due to moisture on population of BSF larvae. The compost will later be tested for seed germination. It is concluded that BSF larvae can be used in animal and household waste management.

PROCESSING OF WATERMELON WASTE FOR BIOETHANOL PRODUCTION USING BIOREFINERY PRINCIPLES

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The conversion of Watermelon as biodegradable waste into biobased economy by using biorefinery principles is other effective steps to reduce the green house gas emission, minimize the material waste and also provide a fuel (bioethanol) of low prices. Response Surface Methodology based optimization was used for hydrolysis of conditions with Sodium hydroxide. The significant amount of reducing sugars (5.11±0.02) was obtained at the end of 0.1% Sodium hydroxide (NaOH) hydrolysis at 80°C for 45 min. After detoxification with 2.5% charcoal, two yeasts *Metschnikowia* sp. (Y31) and *Metschnikowia cibodasensis* (Y34) were used to ferment the hydrolyzates. The significant ethanol yield (%v/v) 2.65±1.53 obtained with *Metschnikowia cibodasensis* (Y34) on day 3 and 2.12±1.23 with *Metschnikowia* sp. (Y31) at day 7 after fermentation respectively. The study shows the hopeful expectations for biobased economy from that biodegradable waste on a large scale.

ANTIBACTERIAL ACTIVITY OF EXTRACT AND BIOGENIC SILVER NANOPARTICLES OF *BERBERIS LYCIUM* ROYLE**Tafail Akbar Mughal, Shaukat Ali* and Saiqa Andleeb***Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan**Microbial Biotechnology and Medical Toxicology Laboratory, Department of Zoology,**The University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan**Corresponding Author: shuakatali134@yahoo.com

There are many antibiotics available in market but bacterial resistance against these antibiotics is increasing day by day, hence antibiotics have lost their efficacy up to much extent. So it is the need of hour to find another antibacterial alternate. Biogenic silver nanoparticles are effective in this regard. Objectives were (i) Biogenic synthesis of silver nanoparticles by using *Berberis lycium Royle* (ii) Characterization of AgNPs (iii) Investigation of antibacterial activities against human-associated bacterial pathogens viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* through agar well diffusion method, MTT assay, and anti-biofilm assay. Characterization: UV-viz spectrophotometry, Scanning Electron Microscope and Fourier-transform infrared spectroscopy were used for the characterization of biogenic nanoparticles. Antibacterial activity: For antibacterial activity of extract and silver nanoparticles agar well diffusion method with different concentrations of plants was used. Biofilm formation was measured using the microtiter dish assay system, MTT assay was used for estimating the viability of bacterial Cells. Presence of Phytochemicals were also analysed. Silver nanoparticles showed maximum zone of inhibition against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* 7.3±0.3 mm, 6±0.00 mm, 6±0.00 mm, 5±0.00 mm, 6±0.00 mm, 6±0.5 mm and 11±0.00 mm respectively as compared to this very low zone of inhibition showed by these pathogens against extract 6±0.00 mm, 1±0.00 mm, 4±0.00 mm, 3.67±0.3 mm, 4±0.05 mm, 5±0.00 mm and 8±0.00 mm respectively. Cell viability assay and antibiofilm assay supported the results of agar well method. Biomolecules e. g tannins, alkaloids, free amino acids, quinones, phenols, terpenoids, steroid and glycosides were found in *Berberis lycium Royle*. It is concluded that b-AgNPs of *Berberis lycium Royle* have antimicrobial activity against tested human pathogens. These nanoparticles can be used to overcome antibiotics resistance problem.

DEVELOPEMENT OF WATERMELON BASED BIOREFINERY FOR BIOETHANOL PRODUCTION**Atika Batool, Nadia Yousaf and Asma Chaudhary***Division of Science and Technology, University of Education Township, Lahore**Corresponding Author: nadiayousaf34@gmail.com

As biodegradable waste usage of watermelon in biorefineries is other hopeful step for the production of cheapest bioethanol. The study shows ethanol yield from dry Watermelon peels by effective fermenting microorganism. For optimization of hydrolysis conditions with Sulphuric acid Response Surface Methodology was used. The maximum amount of reducing sugars (5.70±0.22) was obtained with 1% Sulphuric acid (H₂SO₄) hydrolysis at 50°C for 60 min. After detoxification with 2.5% charcoal, two yeasts *Metschnikowia* sp. (Y31) and *Metschnikowia cibodasensis* (Y34) were used to ferment the hydrolyzates. The significant ethanol yield (%v/v) 2.49±0.02 was obtained with *Metschnikowia cibodasensis* (Y34) and 2.13±0.02 with *Metschnikowia* sp. (Y31) on day 9 after fermentation respectively. The study provides best use of biodegradable waste in production of ethanol on a large scale.

ANTIMICROBIAL AND ANTIOXIDANTAL ACTIVITIES OF *ZIZIPHUS JUJUBA* AND *SARACA INDICA* EXTRACT AND ITS DIFFERENT FRACTIONS

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In this study methanolic extract of *Saraca indica* and *Ziziphus jujuba* were screened for its relative total phenolic contents, total antioxidant and antibacterial activities. In the first part of the study antimicrobial activity of *Saraca indica* and *Ziziphus jujuba* and their organic fractions (hexane, chloroform and ethyl acetate) were checked against gram positive (*Ochrobacterium intermedium*, *Enterococcus avium* and *Actinobacteria*) and gram negative (*Enterobacter hormaechei* and *E.coli*) pathogenic bacterial strains. The antimicrobial activity was performed by disc diffusion and Microdilution assays. Plant extracts and their organic fractions showed varied levels of antimicrobial activity against different strains of pathogenic bacteria. Antioxidant capacities of methanolic extracts and their organic fractions (hexane, ethyl acetate and chloroform) were evaluated using SOD, OH and DPPH radical scavenging assays. Folin-ciocalteu assay was performed to detect the total phenol contents and total antioxidant activities of extracts and its fractions respectively. The antioxidant activities in *Saraca indica* and *Ziziphus jujuba* methanolic extracts and their organic fractions were measured by different methods, including 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, OH radical scavenging activity and super oxide anion scavenging activity. Plant extract and its organic fractions showed varied levels of antioxidant activity in different methods of antioxidant test.

NANO ENGINEERING AND TARGETING DRUG DELIVERY OF METHOTREXATE ONTO HELA CELLS THROUGH ANTI-FOLATE RECEPTOR ANTIBODY MEDIATED ENDOCYTOSIS

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Targeting drug delivery has become the novel approaches for the cancer treatment over chemotherapy. The endocytosis and high expression of Folate receptors on cancer/tumor cells can be targeted for drug delivery. Hydrothermal amino group Magnetic Nanoparticles and their conjugation with Folic Acid were prepared. Anti-cancer drug, Methotrexate (MTX), was immobilized further to prepare MNP-MTX, MNP-MTX-F.A nanoparticles and they were verified through Fourier Transform Infrared (FTIR) Spectroscopy. Folic acid conjugated MNPs were used for purification of highly expressed Folate Receptor (FR) on HeLa cells through novel Magnetic Affinity process. A 37 KDa protein on western blot confirmed the purification of Folate Receptor. Antibodies against Folate receptor and Methotrexate were raised in rabbit and mice respectively, for the confirmation of Folate Receptor and MTX on HeLa cells by ELISA and Immunohistochemistry. Anti-Folate Receptor antibodies were conjugated with MNP-MTX forming the distinctive composite MNP-MTX-Ab for targeting drug delivery by means of antibody mediated endocytosis. All the complexes were tested on HeLa cells and cytotoxic analysis using fluorescent microscopy showed that intracellular uptake of MNP-MTX-Ab was higher and was able to destroy cancer cells. Immunohistochemical studies were completed for binding the complex with tumor tissue and the fluorescence confirmed the uptake of MNP-MTX-Ab. This study was helpful in understanding the targeted drug delivery through antibody mediated endocytosis process.

EVALUATION OF KO7 (SEVEN PROTEASE DEFICIENT) ENGINEERED STRAIN OF *BACILLUS SUBTILIS* FOR PRODUCTION OF RECOMBINANT HUMAN INTERFERON ALPHA 2B (RHIFNA-2B)

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Interferons (IFN) are cytokines produced and secreted by virtually all eukaryotic cells in response to stimulation of foreign antigen. *IFNs* stimulate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Expression analysis of *rhIFN α -2b* in *E. coli* gives disadvantages so therefore scientist switch over to express the gene in *Bacillus subtilis* and used *CytI* promoter which is sporulation dependent and therefore any signal peptide was not used. Coding region of *Interferon alpha 2b* gene was amplified through RT-PCR using mRNA extracted from blood as template. The gene was cloned in T/A cloning vector and then confirmation of gene was done by sequencing. Sequence analysis showed that the gene has a total five point mutations at positions 44, 91, 117, 143 and 154. The point mutations at position 91 and 154 were silent i.e. the amino acid sequence was not affected but mutations at positions 44, 117 and 143 changed the amino acid sequence. At position 44 Methionine changed into Valine, at position 117 aspartic acid changed into glycine and at position 143 Arginine changed into Glycine. Further the gene was subcloned in *Bacillus* shuttle vector pSTAB under control of *CytI* promoter and transformed in *E. coli* (DH5 α). After confirmation, the recombinant plasmid pSTAB- *IFN α -2b* was transferred in *Bacillus subtilis* KO7 strain. Transformants were analyzed for gene expression after five days after incubation in NBG medium. The expression of *IFN α -2b* was very low if it was there as no prominent band of about 19KDa was observed on 15% SDS-PAGE.

PROCESS OPTIMIZATION FOR THE ENHANCED PRODUCTION OF ENDOGLUCANASE BY LOCALLY ISOLATED FUNGAL STRAIN USING SUBMERGED FERMENTATION

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Endoglucanase has a wide range of applications in various industries like pharmaceuticals, textile, detergents and animal feed. The present study revealed the selection of suitable fungal culture for endoglucanase production. Sixteen fungal strains were isolated from different samples like bread, soil, dung and lemon etc. The strains were initially selected qualitatively on berg medium on the basis of cellulose hydrolyzing zone and screened quantitatively for enzyme production in shake flasks. The strain showing highest cellulytic potential was identified and assigned the code *Aspergillus niger* ABT11. The six different fermentation media were evaluated for CMcase and Fpase production by this strain. Among all the tested media, M2 containing 2g Wheat straw and 50 ml of Mandel and Sternberg's mineral medium comprising (g/l): 1.4 (NH₄)₂SO₄; 2.0 KH₂PO₄; 0.3 CaCl₂; 0.0003 MgSO₄. 7H₂O; 0.005 FeSO₄.7 H₂O; 0.0016 MnSO₄. H₂O; 0.0014 ZnSO₄.7H₂O; 0.002 CoCl₂ produced maximum enzyme production. The influence of different physicochemical parameter like incubation time, temperature, initial pH and volume of media was also optimized. The optimal enzyme production was obtained at 72 h, 40°C pH 5.0 and 100ml of volume. In addition to this glucose at the concentration of 2.5% as carbon source, ammonium nitrate (1.0%), were also optimized and gave maximum enzyme production (16.82U/ml).

PROTEASE PRODUCTION FROM MICROBES ASSOCIATED WITH CONTAMINATED SOURCES THROUGH SUBMERGED FERMENTATION

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Proteolytic bacteria are most important for industries such as food and fermentation. The main aim was the production of alkaline protease having characteristics properties and applicable as additive in detergent industries as well dehairing agent in leather processing. *Bacillus subtilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were isolated from different waste soil sources viz., milk waste, slaughter house waste, engine oil waste and sludge soil waste. All isolates were screened for proteolytic activity using alkaline skim milk agar and casein agar plates. Optimization of fermentation medium for maximum protease production was performed by twelve different productions media with different carbon (glucose, fructose and lactose) and nitrogen (peptone and yeast extract) sources, at 37°C after 24, 48, 72 and 96 h incubation periods. Among these strains *Staphylococcus aureus* (f2) showed highly significant proteolytic activity (1007.82 ± 23.20 U ml⁻¹) with Horikoshi medium 2 (HMA2) after the fermentation period of 48 h at 37°C whereas *Bacillus subtilis* (H2) also showed highly significant protease activity (902.44 ± 14.66 U ml⁻¹) in the presence of HMA2 after 72 h of fermentation. It was concluded that the optimum conditions for protease production were found to be 37°C at pH 10 with HMA2 medium. Dehairing and blood stains were actively removed by proteases produced by all microbes. The current research revealed that the waste products can be used as a source for alkaline protease production.

BENCH-SCALE FERMENTATION FOR SECOND GENERATION ETHANOL AND HYDROGEN PRODUCTION BY *CLOSTRIDIUM THERMOCELLUM* DSMZ 1313 EMPLOYING SUGARCANE BAGASSE

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Exploration of alternate energy resources substituting conventional fossil fuels is the real challenge globally. To realize the large-scale production of eco-friendly biofuels such as bioethanol and biohydrogen, it is of prime importance to develop fermentative processes employing low-cost lignocellulosic bio-wastes as substrates. Current investigation accentuates the thermophilic fermentative conversion of sugarcane bagasse into bioethanol and biohydrogen by using *Clostridium thermocellum* DSMZ1313, a renowned thermophilic cellulolytic bacterium. Initially, elucidation of the optimized levels of some influential fermentation factors for bioethanol and biohydrogen productions including crystalline cellulose, corn steep liquor (CSL), ferrous sulphate (FeSO₄), magnesium chloride (MgCl₂), incubation pH and incubation time was done by using Taguchi OA experimental design. Comparable biofuels' yields were obtained when cellulose was substituted with 2% H₂SO₄ pretreated SCB in the fermentation medium. When free cell batch fermentations were further up scaled for production of bioethanol and biohydrogen in bench-scale stirred-tank bioreactor under aseptic conditions, 11.77% improved ethanol and 2-fold higher volume of hydrogen gas were obtained: The production of bioethanol and biohydrogen employing sugarcane bagasse directly as fuels' feed by *Clostridium thermocellum* DSMZ 1313 has appeared a potentially sustainable recourse to meet the remarkably high energy demands.

APPLICATION OF OZONE IN COTTON BLEACHING USING SURFACTANTS TO REDUCE WATER POLLUTION

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The effect of three types of surfactants on the efficiency of ozone bleaching process has been studied in this paper. The results show that anionic surfactant (0.01 g/l) gives the best results in terms of absorbency (1.07 seconds), whiteness (W_{CIE} 64.53) and bursting strength (92lb) at an ozone dose of 10g/h, pH 5 and ozone exposure time of 45 minutes at room temperature. The pollution load of ozone bleaching effluent even after adding surfactant is far less than conventional bleaching effluent. Statistical analysis of the experimental data using analysis of variance confirmed that surfactant addition significantly improved the ozone bleaching performance.

BIODEGRADATION OF REACTIVE RED S₃B BY LIGNOLYTIC ENZYMES OF *FOMITOPSIS PINICOLA*

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The present study was done to figure out the ability of lignolytic enzymes of *Fomitopsis pinicola* to detoxify wastewater. Textile industries use many dyes that are left untreated and eventually cause water pollution. Reactive red S₃B was used to check the biodegradation rate of lignolytic enzymes by *F. pinicola*. Optimization of degradation process was done by using Response Surface Methodology. About 93% biodegradation was observed after initial optimization of different cultural conditions. Biodegradation was increased upto 4% by addition of carbon and nitrogen sources. The maximum activities of laccase, manganese peroxidase and lignin peroxidase were found to be 298 U/ml, 212 U/ml and 268 U/ml, respectively. Maximum purification of enzymes was observed at 50 to 60% salt concentration and 9th elution of Sephadex G100 column. Optimum pH for laccase and lignin peroxidase was 4 and of manganese peroxidase was 5. Optimum temperature for laccase and lignin peroxidase was 30°C and for manganese peroxidase was 35°C. The values of K_m were 12.07, 13.5 and 17 μ M/ml/min for laccase, MnP and LiP respectively. The values of V_{max} were 714.2, 833.3 and 1028.57 Mm for Laccase, MnP and LiP respectively. It can be suggested from the current study that dyes can be degraded by lignolytic enzymes of *Fomitopsis pinicola*. By providing optimum conditions the rate of biodegradation can be increased. This approach can be used to treat wastewater containing harmful dyes to overcome the water pollution caused by textile industries.

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF LIGNOLYTIC ENZYMES BY *ASPERGILLUS FLAVUS*

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Ligninolytic enzymes are employed for the production of second generation biofuel to minimize fuel crisis. Additionally, it plays a crucial role in global carbon cycle and a variety of applications in food,

agriculture, paper and textile industries. For large scale production of ligninolytic enzymes production microorganisms can be cultured on lignocellulosic wastes. In the present study, *Aspergillus flavus* potential to produce ligninolytic enzymes was investigated by optimizing various cultural and nutritional conditions. Proximate analysis of various agro-wastes was done and *Platanus orientalis* was selected as substrate as it has more lignin contents. Physical parameters such as temperatures, fermentation period, pH, inoculums size, substrate mesh and substrate size were optimized for solid state fermentation (SSF) through response surface methodology (RSM) with a central composite design (CCD). The optimum SSF conditions were: temperature 32°C, fermentation period 120 hours, pH 4.5, inoculums size 3.5mL, substrate mesh 80, substrate size 7g. Maximum purification of laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) was achieved with 50%, 60% and 40% ammonium sulfate respectively and Gel filtration chromatography had increase purification many folds. Laccase has 35 optimum temperature, 4.5 pH, 0.289 mM Km and 227.27 uM/ml/min Vmax. Manganese peroxidase has 30 optimum temperature, 5.5 pH, 0.538 mM Km and 203.08 uM/ml/min Vmax. Lignin peroxidase has 30 optimum temperature, 3 pH, 2mM Km and 2000 uM/ml/min Vmax. Protein concentration found in crude extracts and partially purified enzymes respectively: laccase 1.78 and 0.71 mg/mL, MnP 1.59 and 0.68 mg/mL, LiP, 1.70 and 0.69 mg/mL.

STUDY OF EXTRACELLULAR ENZYMES INVOLVED IN BIODEGRADATION OF VAT DYES BY *P. BETULINES*

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Environment is entirely exposed to dyes either by effluents from synthetic plants or textile industries. About 2-50 percent dyes are released into textile effluents during their application process. Reactive dyes in water bodies increase biological and chemical oxygen demand reduce germination rate of plant biomass. In human beings respiratory problem, nasal problems, mutagenicity, teratogenicity are arising due to presence of reactive dyes in water bodies. Algae, bacteria, yeast, fungi and actinomycetes have been reported for their potential to decolorize reactive dyes. A study was conducted to evaluate the decolorization potential of brown-rot fungus *P. betulines*. Decolorization of reactive4 under initial optimization conditions showed 56.45% degradation following pH 6, inoculum size 6mL, temperature 32.5, 0.05% dye concentration and incubation period 5 days. Reactive5 biodegradation under initial optimization conditions showed 57.76% degradation following pH 6, inoculum size 6mL, temperature 32.5, 0.03% dye concentration and incubation period 5 days. Interaction among different parameters was evaluated by JMP software under central composite design. Study of lignolytic enzymes produced during decolorization of reactive 4 showed following activities. Laccase 19.44U/mL, manganese peroxidase 104.36U/mL and lignin peroxidase 23.65U/mL. Study of lignolytic enzymes produced during decolorization of reactive 5 under initial optimization conditions showed laccase activity 53.8U/mL, manganese peroxidase 120U/mL and lignin activity was 29.03U/mL. Using optimized conditions, addition of carbon and nitrogen sources enhanced decolorization of reactive dyes. 99% decolorization occurred in reactive4 with 0.03% glucose, urea and 0.01% fructose; while 97% decolorization occurred in reactive 5 by 0.03% glucose, fructose and 0.05% urea. Lignolytic enzyme assays after final optimization for biodegradation of reactive 4 showed maximum laccase activity 2.22U/mL, manganese peroxidase 4.72U/mL and lignin peroxidase 9.67U/mL. In case of reactive 5 laccase activity is 2.77U/mL. MnP and lignin peroxidase activities 6.54 and 9.67U/mL respectively. Characterization performed showed maximum activities of laccase, MnP and lignin under following conditions. Enzymes worked best at 35°C under pH 5.5, 3.5 and 3 respectively. Km and Vmax for laccase is 99mM and 20.04uM/mL/min, MnP Vmax=20.20uM/mL/min and Km=3.72mM while Vmax and Km for lignin is 23.58uM/mL/min and 9.85mM. Effect of metal ions on lignolytic enzyme activities showed maximum laccase activity in presence of KCl. MnP and lignin showed maximum activities in presence of MgSO₄. Purification of lignolytic enzymes by ammonium sulphate precipitation method showed maximum activities in pellet. Purification by gel filtration chromatography

showed maximum activity of lignin peroxidase (120U/mL). Protein quantification showed presence of protein in sample. The amount of proteins in the sample was found to be 0.2 mg/mL. It is concluded from study that lignolytic enzymes produced by *P. betulines* are effective in decolorization of reactive 4 and 5. *P. betulines* can be used competently for effective removal of colors from textile wastewaters when provided with optimized parameters and nutritional sources.

BANANA WASTE AN EFFICIENT CHEAP SUBSTRATE FOR XYLANASE PRODUCTION

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Xylanases(endo-1, 4 beta -D-xylanohydrolase; EC 3.2.1.8) are widely used in pulp bleaching, in clarification of juices, in improving the digestibility of poultry feed, and in the production of 2nd generation biofuels. The bacterial strain *Bacillus licheniformes* was isolated from fish gut and was screened for its potential to produce xylanase using banana peels powder as substrate. The xylanase medium was optimized by using response surface methodology. The medium components (peels, yeast extract, KH₂PO₄, K₂HPO₄, sodium nitrate, MgSO₄, peptone, NaCl, and ammonium sulphate) were screened employing 12 runs Plackett-Burman design. Four out of nine mediums with significant results were optimized for their maximum concentration using Central-composite design. The banana peels (1.5%), sodium nitrate(0.38%), peptone(0.80%), and MgSO₄(0.77%) gave maximum enzyme production. The statistical model dictated significance (P 0.001) of the model. The R² value was found to be 0.82. The optimum temperature (30°C) and pH (3) yielded xylanase upto 0.33U/ml and 2.24U/ml. The 2% inoculum size and 23 hours of incubation were optimum for maximum xylanase production. Maximum xylanase activity was recorded at pH 8, 55°C incubation temperature, 2% substrate concentration and 90 minutes of incubation time.

OPTIMIZATION OF AMYLASE PRODUCING BACTERIA BY *BACILLUS CEREUS*

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The aim of present study was to screen the amylase producing strains which had been previously isolated from fish gut. Moreover, the objective was also to optimize the production of amylase from *Bacillus cereus* in a medium by employing Response Surface Methodology. Eight strains of bacteria were revived on nutrient agar and on the basis of clear zones on selected medium, five strains were selected. Enzyme assay was performed for these strains, and on the basis of highest enzyme value (177±0.00U/ml), *B. cereus* was selected. The optimum temperature for *B. cereus* was found to be 37°C (2.5 ± 0.11 U/ml) as compared to 30°C and 45°C. Similarly the optimum pH was found to be 9 (20.48±0.20U/ml) in comparison to pH 5 and 7. The 1% inoculum size showed highest enzyme activity (12.87± 0.25U/ml) as compared to 3% and 5%. When incubation period was optimized, it was observed that 24 hours were an optimum condition for maximum (22.48±0.12U/ml) amylase production in comparison with 48, 72 and 96 hours of incubation. The medium components (starch, NaCl, CaCl₂, K₂HPO₄, MgSO₄, and yeast extract) were screened employing 12 runs Plackett-Burman Design. From 12 experimental runs, 3 significantly affecting parameters (starch, MgSO₄ and yeast extract) were selected. For obtaining the mutual interaction between these variables and optimizing these variables, Box-Behnken Design (15 experimental runs) was employed and the combined effect of starch (0.5%), MgSO₄ (0.1%) and yeast extract (0.275%) showed the highest amylase production. The statistical model dictated the significance (P<0.001) of model. The R² value was recorded up to 0.90. In present study, the amylase produced by *B. cereus* showed

highest activity at pH 10 (34.08 ± 0.93) in comparison with pH 4, 5, 6, 8, 9, 10 and pH 11. The optimized temperature for amylase activity was recorded 60°C (16.06 ± 0.7). When variable substrate concentrations were examined, it was found that 0.5% substrate concentration was optimum (60.06 ± 0.7 U/ml) for amylase activity in comparison with 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, and 3.5% substrate concentration.

OPTIMIZATION FOR TANNASE PRODUCTION BY *KLEBSIELLA OXYTOCA* IN SOLID-STATE FERMENTATION OF MUSAMBI PEELS

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Presence of polyphenols such as tannins in agro industrial wastes suggests that they could be utilized for the production of tannase. The present study focused on optimization of the production parameters for tannase employing agro-industrial waste. Ten bacterial isolates from fish gut were revived on tannin-agar plate and four were selected on the basis of clear zones. Enzyme assay was performed for these four strains and on the basis of highest enzyme value (24.52 U/ml), *Klebsiella oxytoca* was selected. One factor at a time method was used to select the medium ingredients (musambi peels, trypton, potassium nitrate, potassium chloride). Tested variables for maximizing enzyme production were as follow: musambi peels 2%, trypton 0.2%, potassium nitrate 0.05% and potassium chloride 0.1%. The optimum pH was found to be 3 (9.93 ± 2.99 U/ml) in comparison with pH 7 and 9. Similarly, the optimum temperature for *K. oxytoca* found to be 30°C yielding up to 10.06 ± 2.46 U/ml as compared to 37°C and 40°C . The 1% inoculum size showed highest enzyme production (16.70 ± 4.19 U/ml) as compared to 3% and 5% inocula. When incubation time was optimized, it was observed that 24 hours was optimum for maximum amount of tannase (16.76 ± 1.78 U/ml) in comparison with 48, 72 and 96 hours incubations. In the present study, *K. oxytoca* showed highest tannase activity (14.88 ± 1.50 U/ml) at pH 5 in comparison with pH 4, 6, 7, 8, 9, 10 and 11. The optimized temperature for tannase activity was recorded 40°C (20.34 ± 3.70 U/ml). The optimized incubation time for tannase activity was 30 minutes (30.60 ± 1.16 U/ml). When variable substrate concentration were examined, it was found that 1.5% substrate concentration was optimum for tannase activity (30.81 ± 2.52 U/ml) in comparison with 0.5%, 1%, 2%, 2.5% and 3% substrate concentrations.

STATISTICAL OPTIMIZATION OF *BACILLUS SAFENSIS* FOR PROTEASE PRODUCTION

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The physical and medium parameters influencing protease production by *Bacillus safensis* isolated from the fish gut were optimized. The selected strain yielded maximum protease at 30°C , pH 9 with 1% inoculums size following 3 days of in submerged fermentation. After employing 12 runs of Plackett-Burman Design for the medium components (casein, KH_2PO_4 , K_2HPO_4 , MgSO_4 , NaCl, glucose and peptone). Of these ingredients, casein, KH_2PO_4 and peptone were found significant on the basis of pareto chart. The mutual interactions among selected variables using Box-Behnken Design analyzed and results dictated that medium containing 1.4% casein, 0.05% KH_2PO_4 and 0.575% peptone had maximum protease production. The R^2 value (0.99) indicated accuracy of the model. Maximum enzyme stability was found at 40°C temperature, pH 8 with 0.65% substrate concentration.

SCREENING AND OPTIMIZATION OF FERMENTATION MEDIUM FOR LYSINE PRODUCTION BY *CORYNEBACTERIUM GLUTAMICUM* IIB646**Amjed Hussain^{1*}, Hamid Mukhtar² and Ikram-Ul-Haq²**¹*Department of Zoology University of Kotli-11100, Azad Jammu and Kashmir*²*Institute of Industrial Biotechnology, GC University Lahore-54000, Pakistan*

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L-lysine is an essential amino acid and microorganisms are able to synthesize both essential and non-essential amino acids through fermentation process. Fermentation contributes 80% of the L-lysine that is annually produced worldwide. The present work describes the production of L-lysine from *Corynebacterium glutamicum* IIB646 through submerged fermentation process. Fifteen different fermentation media were used and screened in this study for L-lysine production. Out of these fifteen media, FM:13 was found a best one for maximum 5.5 g/L production of L-lysine. The medium contained glucose, ammonium sulphate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, calcium carbonate, thiamine HCl, biotin, manganese chloride tetrahydrate and casamino acids. This medium was further supplemented with different nitrogen and carbon sources to increase the L-lysine production. Glucose, as best source of carbon and ammonium sulphate as a best nitrogen source was optimized. Finally 7.4 g/L of L-lysine was produced after 72 hrs incubation in FM:13 medium supplemented with 7% glucose and 2.5% ammonium sulphate.

THERAPEUTIC POTENTIAL OF GOLD NANOPARTICLES SYNTHESIZED FROM LEAVES EXTRACT OF *SOLANUM NIGRUM* FOR LIVER DAMAGE *IN VIVO***Aneela Sohail^{1*}, Muhammad Tariq¹, Badar ul slam¹, Afshan Afreen² and Tayyibah Tariq²***Department of Biotechnology, Mirpur University of Science and Technology, 10250 Mirpur, AJ&K*

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Metal nanoparticles especially gold nanoparticles (GNPs) prepared from extracts of natural sources such as plants have been extensively exploited in last decade for many biomedical applications such as antimicrobial, drug delivery. The present study was an attempt to investigate the hepato-protective, antimicrobial and antioxidant activities of GNPs synthesized by using extract of leaves of *Solanum nigrum*. GNPs were characterized by UV-Visible, XRD, SEM and FTIR and their antimicrobial and antioxidant activities were evaluated *in vitro*. Further, *in vivo* hepato-protective activity of GNPs was assessed against carbon tetrachloride (CCl₄) induced liver injured rabbits. For this purpose, rabbits were divided in two groups (n=6) viz control group which was given normal saline only while treatment group was given GNPs @ 0.1 mg/kg/day for two weeks. After two weeks, blood was taken and evaluated for ALP, ALT, AST and bilirubin. Moreover, animals were sacrificed and their liver were excised for histopathology to observe morphological changes in liver structure. The results of SEM, FTIR, UV-Vis and XRD confirmed the formation of GNPs from the extract of leaves of *Solanum nigrum*. GNPs showed good antibacterial and antioxidant activities. The treatment of liver injured rabbits with Gold nanoparticles resulted in reduction in the values of ALP (200±17.4), ALT (93.4±5.7), AST (63.5±2.7) and Bilirubin (0.81±0.07) as compared to control group. Moreover, histopathological examination showed improvement in the morphology of liver of GNPs treated rabbits as compared to control group. The present study showed antibacterial and antioxidant activities of gold nanoparticles from the extract of leaves of *Solanum nigrum*. Moreover, GNPs also showed ameliorative potential for liver damage.

CADMIUM AND CHROMIUM REMOVAL ABILITY OF SELECTED STRAINS OF *LACTOBACILLUS* spp.**Sania Nadeem, Aneela Muneer, Ayesha Riaz, Saleha Noureen and Najma Arshad***Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore-54590, Pakistan*

Heavy metal toxicity is common in industrialized areas. A bioremediation process was used to remove heavy metals with the help of symbiotic intestinal microbes. A total 08 Lactic acid bacteria isolates named

(An16, An10, An20, An28, An30, R, A, and *Lactobacillus acidophilus* ATCC4356 were used in the study. The tolerance and removal ability of selected strains was investigated against Cr and Cd. Potassium dichromate and Cadmium chloride were used as a source of metal. Different concentrations of these salts 250-2000 µg/ml were supplemented in MRS medium. All the strains showed growth up to 1300µg/ml and 1250 µg/ml of Cr and Cd respectively. Metal removal activity was observed at different time intervals (0 hour, 2 hours and 6 hours). Chelation process was done on hot plate at 95°C. Supernatants and pellets were examined for amount of metal removal through atomic absorption (AAS). Strain “A” from cow and “An28” from camel exhibited metal removal activity, which was equivalent to 75%=156.25mg/h/10¹⁰cfu and 37%=77.08mg/h/10¹⁰cfu for chromium and 70%=145mg/h/10¹⁰cfu and 28%=58.3mg/h/10¹⁰cfu for cadmium respectively. Further studies targeting the genes involved in metal removal and *in vivo* trials on efficiency of metal removal are in progress.

2. CELL BIOLOGY AND GENETICS

MOLECULAR ANALYSIS OF NON- SYNDROMIC AUTOSOMAL RECESSIVE CONGENITAL CATARACT IN AN AFFECTED PAKISTANI FAMILY

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Cataract term is used for cloudiness of the crystalline eye lens. Non-syndromic autosomal recessive congenital cataract (ARCC) is mainly inherited through consanguineous marriages. If genes that encode lens proteins and growth regulators are mutated then normal protein cannot be formed, this leads to disturbed lens protein structure and function. If such mutated copy of gene is inherited in the child from both parents, then ARCC can appear. The study was conducted to identify genetic reason of ARCC in an affected consanguineous Pakistani family. Linkage analysis through STR markers to detect mutated gene responsible for this genetic defect was the main objective of the research. To meet the objective, blood samples from the affected family (CAT-4) were collected followed by DNA extraction. PCR was performed with polymorphic microsatellite markers for all 15 autosomal recessive congenital cataract loci. PCR product was analyzed on 8% PAGE for genotyping. Linkage was observed with microsatellite marker D22S686 (13.6 cM) on 22q11 locus. Homozygosity with this microsatellite marker was only observed in affected individuals. The linked microsatellite marker, flanked by markers D22S689, D22S427 and D22S926 contains β -crystalline gene cluster. The flanked markers also showed linkage. The family was further subjected to DNA sequencing of *CRYBB3* gene for mutation screening. However, no significant mutation was found in it indicating the cause of ARCC. Furthermore, DNA sequencing of other β -crystalline genes is being done as they are also present at the same region.

A STUDY OF RECURRENT GLIOBLASTOMA MULTIFORME (A DESTRUCTIVE PRIME BRAIN TUMOR) AND ITS TREATMENT WITH TEMOZOLOMIDE, (AN ALKYLATING AGENT)

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Glioblastoma multiforme (GBM) is a maximum destructive prime brain tumor. Glioblastoma multiforme signifies 15 to 20% of all the principal cancers and it is grade 4 tumor. The general sign and symptoms of this disease are nausea, headache and conditions which is normally in the case of stroke. Temozolomide (TMZ) is an oral alkylating agent and chemotherapeutic drug which is taken orally for the treatment of cancerous brain tumor. Temozolomide is not active until it is converted at physiologic pH to the active form, 5-(3-methyltriazene-1-yl) imidazole-4-carboxamide (MTIC). Dose-dense chemotherapy is defined as chemotherapy in which drugs are administered to the patients for relatively less time than the actual time span of the treatment. Dose-intense chemotherapy is also employed in the case of GBM. Most importantly and greatly used is dose-dense temozolomide in the case of GBM relapse. This is used to improve the average existence rate of the patients. Nonstop exposure of cancer cells to temozolomide begins the diminution of O6 -methylguanine-DNA methyltransferase (MGMT). MGMT depleting agents were developed like O6 -benzyl guanine which causes the depletion of MGMT. To diminish MGMT faster nitrosoureas and temozolomide work mutually. The amount of TMZ which is accepted and permitted is 150 to 200 mg/m² per day. To cause the diminution of O6-methylguanine-DNA methyltransferase which is responsible for the increase in cytotoxic activity additional amount or dosage of TMZ is given. By the use of TMZ dose-intense regime, we analyze efficiency and protection of patients suffering from GBM relapse. Dose-dense chemotherapy is applied continuously for a better outcome. Continuously dose-dense chemotherapy is also applied in the case of temozolomide. In the treatment of patients having high-grade glioma this continuous dose-intense temozolomide is utilized for better results. In the case of chemotherapy, various side effects occur which include nausea, vomiting, pain, fever, loss of hair, anemia, loss of appetite, constipation, diarrhea etc. To reduce these side effects chemotherapy is

combined with cannabinoids. We conclude from this study that TMZ is 60% more efficacious for recurrent Glioblastoma. In all the groups it is not as much efficient but still gave positive results. Further researches are still going on. This is the small report on the study of recurrent GBM and its treatment with TMZ.

PHOTOCYTOTOXIC EVALUATION OF *TECOMA STANS* AND *NARCISSUS TAZETTA* MEDIATED SILVER NANOPARTICLES AGAINST RHABDOMYOSARCOMA CELL LINE

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Nanobiotechnology combines biological principles with physical and chemical approaches to produce nanosized particles with specific properties in eco-friendly way. Photodynamic therapy along with nanotechnology is gaining tremendous importance with enhanced efficacy. The present work was aimed to evaluate methanolic extracts and nanoparticles of two selected plants (*Tecoma stans* and *Narcissus tazetta*) for their Photodynamic, anticancer potential. GC-MS analysis of extract of all plant parts showed presence of biologically active metabolites. Silver nanoparticles and extracts showed decreased % cell viability against rhabdomyosarcoma cell line. The result of Photodynamic study showed that efficacy of photosensitizer is enhanced when nanoparticles are used as an adjunct. The silver nanoparticles (AgNPs) were found to be spheroidal in shape with size ranging between 15-100nm and showed peaks in specific regions of Fourier-transform infrared (FT-IR) spectra. The current study showed that these silver nanoparticles can be used to enhance Photodynamic cytotoxic potential. These nanoparticles due to their biological efficacy can be further used to establish medicinal potential of selected plants.

DETERMINATION OF IRS-1 IN MCF-7 AND MDA BREAST CANCER CELL LINES BY COMBINING PROTEOMIC AND BIOINFORMATICS APPROACHES

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The involvement of post translational modifications (PTMs) in cell metabolism is a well-known phenomena, and defining signaling pathways, where aberrant regulation contributes to insulin resistance and oncogenesis, is a timely endeavor. The insulin receptor substrate protein 1 (IRS-1) is a key regulator in the insulin signal transduction, and has shown to behave aberrantly in diseases such as diabetes and cancer. Nuclear IRS-1 has already been associated with breast cancer cells and has been detected near the site of damaged DNA in medullablastoma cells. The function of IRS-1 is regulated by phosphorylation on its Ser/Thr residues. Phosphorylation of IRS-1 is involved in malignant transformation, and specifically phosphorylated Ser has been shown to be involved in the development of cancer. The present study was designed to determine the presence phosphorylated IRS-1 in MCF-7 and MDA human breast cancer cell lines by combining proteomic and bio-informatic approach. IRS-1 in different breast cancer cell lines MCF-7 and its metastasis MDA cells was done by SDS-Page and western-blotting. It was determined that IRS-1 was phosphorylated in both cell lines being highest in MCF-7 as compared to MDA cell line. These results suggest that phosphorylated IRS-1 could be a potential target for anticancer therapeutic agents of breast cancer therapy and in the near future targeted drug designing may be possible.

GENETIC AND EPIDEMIOLOGICAL STUDIES OF HEPATOCELLULAR CARCINOMA PATIENTS IN A HOSPITAL POPULATION OF FAISALABAD CITY AND THEIR BLOOD PROTEIN PROFILE

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The prevalence of hepatocellular carcinoma (HCC) has been increasing throughout the world including Pakistan from the last few decades but data on the current estimate of hepatocellular carcinoma prevalence in Pakistan was not updated. Current study was carried out to estimate the incidence, prevalence and risk factors responsible for HCC and its genetical basis in hospital population of Faisalabad city. The genetic and epidemiological study was performed on hepatocellular carcinoma patients at Allied Hospital and District Headquarter Hospital, Faisalabad by using a standardized questionnaire for patients. The blood protein profile of hepatocellular carcinoma patients was also analyzed through the process of SDS-PAGE. Out of 1000 subjects of hepatocellular carcinoma 57.1% were male and 42.9% were female. Overall, four types of liver disorders were encountered with descending order 48.6% (decompensated chronic liver disease DCLD), 39% (HCC), 6.3% (multifocal hepatocellular carcinoma MFHCC) and 6.1% (decompensated chronic liver cirrhosis DCLC). Generalized mean age of diagnosis was 52.78 ± 0.33 years whereas in case of males it was 52.52 ± 0.46 years and in females 53.14 ± 0.47 years. Generalized mean age at present was 54.01 ± 0.32 years whereas in case of males it was 53.65 ± 0.45 years and in females 54.49 ± 0.46 years. Total subjects were placed into 8 age groups starting from 11-20 to 80+ years. Age group 51-60 years was more prone to disease (33.7%). There were 55.7% rural and 44.3% urban patients belonging to 46 surnames, more prevalent was Arain (20.7%) followed by Jutt (18.1%) and Rajpoot (16%). Majority of subjects were married 96.8%. Maximum subjects were placed in 2nd birth order (25%) followed by 1st birth order (24.7%) and 3rd birth order (20.4%). Majority of patients were in unskilled category (47%) with school level education (50.3%), and lower socioeconomic status (57.4%). There were 22.2% smokers, 5.5% passive smokers, 7.2% ex-smokers and 65.1% non-smokers. Majority of patients had mixed type of diet (93.6%), drank pump water (75.6%) and used community barber shop for shaving (63.05%). Associated complications in HCC patients included hepatitis C (94.7%), cirrhosis of liver (47%), diabetes (30.9%), hepatitis B (4.4%), obesity (1.5%) and nonalcoholic fatty liver disease (0.5%). Majority of HCC subjects have the previous history of hepatitis C (50.1%) followed by cirrhosis of liver along with hepatitis C (43.3%). Majority of subjects (47.1%) were in first cousin parental relationship. Overall 26.5% cases showed positive family history. Majority affected relatives were first degree relatives (84.44%) followed by second degree relatives (15.56%). Generalized F-value was 0.061. The blood protein profile of HCC patients revealed that average albumin concentration was low (2.9 ± 0.01 g/dl), average globulin concentration was high (3.5 ± 0.007 g/dl) and albumin/globulin ratio was low (0.8 ± 0.005 g/dl) as compared to their normal ranges. The eight protein subunits of molecular weight 250, 130, 95, 66, 55, 45, 28 and 15 KDa were observed as polymorphic in nature and showed variations. This study has suggested certain endorsements to reduce the prevalence rate of hepatocellular carcinoma.

CONSANGUINITY AND ITS EFFECTS IN THE POPULATION OF DISTRICT FAISALABAD, PAKISTAN

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The world is progressing but some traditions and rituals are passing from generation to generation never changed. Consanguineous marriages are one of the old and largely practiced tradition and continuing from human origin on earth. In thousand million people of the world, about 20% to 50% marriages are consanguineous. Consanguinity enhances the chances to carry homozygous deleterious recessive alleles which result in different genetic disorders. The present study was done to discover the prevalence and effects of consanguinity on human health in district Faisalabad of Punjab, Pakistan and inbreeding coefficient was

calculated. When compared with other districts of Punjab it was observed the regional heterogeneity demanding further studies.

ANALYSIS OF GENETIC DIFFERENTIATION AMONG POPULATIONS OF *Aedes Aegypti* IN DISTRICT LAHORE USING RAPD/MICROSATELLITE MARKERS

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An experiment was designed to estimate the genetic variability in *Aedes aegypti* populations in different areas of district Lahore, using Random Amplified Polymorphic DNA and Microsatellite markers. *Aedes aegypti* mosquitoes were collected by using standard techniques (dippers) from selected areas of urban and rural sites and reared separately in the laboratory to extract DNA individually. DNA concentration was measured by using electrophoresis standards after the extraction. Ten decamer random primers (GL Decamer A-01 to GL Decamer A-10) were selected for Polymerase Chain Reaction (PCR). The PCR products were analyzed using 1.5% agarose gel electrophoresis. The amplification profile data showed monomorphism and polymorphism between samples of different regions of same district. The primer A-04 showed a clear polymorphic banding pattern in different *Aedes aegypti* samples. Based on the inter- and intra-populations polymorphic data from the current findings, it is concluded that *Aedes aegypti* populations were slightly genetically different in selected areas.

COMPARATIVE STUDY OF GENETIC POLYMORPHISM OF MOSQUITO POPULATION FROM URBAN AND RURAL AREAS OF SIALKOT DISTRICT USING DNA MARKERS

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A comparative study was conducted to observe genetic polymorphism among mosquito populations from urban and rural areas of district Sialkot. Mosquitoes collected by using standard techniques (dipper and netting) from different sampling sites of both urban and rural areas were reared separately in the laboratory. The mosquitoes were identified up to genus level, and their DNA was extracted using Salt- and CTAB- extraction protocol. In order to estimate the level of genetic variability among different mosquito populations, Random Amplified Polymorphic DNA analysis technique was used. Random decamer primers were selected and amplified using Polymorphic Chain Reaction (PCR). It was noted that ten arbitrary RAPD primers from Genelink showed highly polymorphic banding profile, therefore indicating the high level of genetic variations in the population of mosquitoes. The current findings would be helpful to design a better program to combat mosquito-borne diseases in and around the vicinity of Sialkot in future.

A HOMOZYGOUS C.1131G>A MISSENSE MUTATION IN BBS9 GENE MANIFESTING AUTOSOMAL RECESSIVE BARDET-BIEDL SYNDROME IN CONSANGUINEOUS KASHMIRI FAMILY

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Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathic genetic disorder in humans. It is a

multisystem disorder and is principally described by visual abnormalities, con-rod dystrophy, eyes exotropia, obesity, polydactyly, hypogonadism, and renal abnormalities. Few additional features of BBS also include delayed motor development, clumsiness, anosmia, ataxia, hypodontia, hearing impairment, hirschsprung disease, cardiovascular and liver disorders. So far 21 genes are reported that cause BBS (BBS1-BBS21). A consanguineous family having clinical symptoms of BBS9 is described in current study. The linkage was established to BBS9 on chromosome 7p14.3 using whole exome sequencing (WES). A splice acceptor site mutation (c.1131G>A) in exon 3 was revealed by Sanger Sequencing.

GENETIC RELATEDNESS IN HATCHERY POPULATIONS OF *LABEO ROHITA* USING SSR MARKERS

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Several exploited freshwater fisheries resources and inappropriate hatchery stock propagation practices are compromising the genetic integrity of distinct local populations. The present study endeavors examine the genetic structure of fish *Labeo rohita* in hatchery populations as influenced by artificial propagation programmes. The fish specimens were collected from the target sites, 30 from each hatchery population originating from different districts of Punjab. Total fifteen microsatellite loci were employed to examine genetic differentiation and population structure of the species within sampling localities. The number of alleles per locus varied between 2 and 7 with an average of 4.0 alleles per locus. Significant deviations from Hardy-Weinberg equilibrium ($P < 0.05$, $P < 0.01$, $P < 0.001$) were observed in about 72% of the total locus-population combination tests with apparent heterozygote deficits. The AMOVA revealed two major components of genetic variation; within-population (93.47%) and among-populations (6.04%). The UPGMA dendrogram clustered the populations onto two major branches, surprisingly departing from their geographical origin. The study points out the factors involved in genetic differentiation and shaping the existing patterns of population structure of the *L. rohita* so as to provide guidelines for conservation strategies and management programs.

ASSESSING THE GENETIC INTEGRITY OF *HYPOPHthalmichthys molitrix* FROM RIVER RAVI BY USING SSR GENOTYPING

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Information regarding the genetic status of aquaculture species is unavoidable for conservation and proper management. Fish genetic integrity is continually being threatened, by anthropogenic interventions at various scales. This proposed study was accomplished to assess the degree of genetic status from wild populations of *Hypophthalmichthys molitrix*. To this end, 30 samples of *H. molitrix* were sampled out from six selected locations of the Ravi River such as Head Balloki, Sidhnai-Mailsi Link, Balloki-Sulemanki Link, Qadirabad-Balloki Link, Marala-Ravi link and Trimmu-Sidhnai Link. To evaluate the allele frequency of Silver carp five polymorphic microsatellite markers (Homo-11, Homo-13, Homo-25, Homo33 and Homo-34) were used. DNA samples were extracted from the fish dorsal muscle by using standard method chloroform: isoamyl alcohol. The values of *FIS* were showed significant differentiation in all six *H. molitrix* populations. UPGMA-Dendrogram results showed that all the six populations formed two major clusters. Genetic distance between all six populations was high to moderate. Mean values of observed heterozygosity (*Ho*) ranged from 0.5400 to 0.6122 while, the mean expected heterozygosity (*He*) values ranged from 0.6330 to 0.7671 for all *H. molitrix* populations. Many population genetic parameters such as genetic distance, allelic richness, loss of heterozygosity, Inbreeding co-efficient, Hardy-Weinberg Equilibrium were studied by using some softwares viz., TFPGA, POPEGENE and F-STAT. This research should be encouraging to evaluate the drastic decline in

genetic integrity of *H. molitrix* populations and would give the valuable data for the improvement of conservation approach.

ASSESSING THE GENETIC STRUCTURE OF HATCHERY PRODUCED *HYPOPTHALMICHTHYS NOBILIS* BY SSR GENOTYPING

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Genetic monitoring of fish population is essential for conservation and management. In hatcheries, fish brood stocks are facing the issues like mismanagement and genetic degradation. To avoid this genetic degradation, it is important to have proper information about genetic diversity of fish stock for optimum exploitation and stock management. In the present study, genetic structure of *H. nobilis* was assessed using by microsatellite SSR markers. The objectives of this research was to assess the extent of genetic diversity of Bighead Carp (*Hypophthalmichthys nobilis*) in different hatcheries of Punjab. For this purpose, sample individual were collected from public Fish Seed Hatchery Satyana Road Faisalabad, Sahiwal hatchery, Sarghodha Nursery and Farooqabad Nursery. Total 30 individuals of subject fish was collected from each hatchery site. Phenol, chloroform and isoamyl alcohol method was used for DNA extraction and confirmed by 0.8% agarose gel. For analysis of genetic variation, were utilized five polymorphic microsatellite loci (Homo11, Homo13, Homo25, Homo33 and Homo34). The target loci were PCR-amplification and PCR product, were resolved on polyacrylamide gel electrophoresis for genotyping. The genotypic were analyzed by using different references, i.e. POPGENE, FSTAT and TFPGA. Various parameters including observed and expected heterozygosity, allelic richness, genetic differentiation were calculated. Observed heterozygosity range between 0.78-0.3328, and expected heterozygosity was 0.5657-0.774794. The significant *Fst* values exhibited genetic differentiation to amongst populations except for FQD - SWL population pair. The calculated genetic distance was found minimum between FSD and SGD populations. This information about genetic structure could be helpful in resolving the genetic issues pertaining to *H. nobilis* re-stocking programs and brood stock management practices.

SCREENING OF THE FAMILIES AFFECTED WITH AUTOSOMAL RECESSIVE MICROCEPHALY SHOWED HOMOZYGOUS NONSENSE MUTATION IN *ASPM* GENE

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Autosomal recessive microcephaly is a heterogeneous genetic disorder characterized by reduced head circumferences and slopping forehead. Affected individuals also show mild to moderate intellectual disability. To date 17 genes have been identified which are involved in autosomal recessive microcephaly. In Pakistani Population *ASPM* and *WDR62* are most frequently involved genes and mutations in these two genes are responsible for MCPH. In present study two consanguineous families were recruited from Sialkot Pakistan. Screening of these two families with STS markers followed by Whole exome sequencing and Sanger sequencing showed c.4802C>G nonsense mutation with protein modification p.S1601X in *ASPM* gene. Thus our study correlates with previous data that *ASPM* gene is major candidate gene responsible for MCPH in Pakistani population.

**IDENTIFICATION AND ANALYSIS OF HUMAN NEUROFIBROMIN GENE 1
FOR STUDYING GENETIC VARIATIONS****Muhammad Waseem Shoaib¹, Saima Jabeen¹, Ammara Masood² and Hira Mubeen^{*2}**¹District Head Quarter (DHQ) Hospital, Faisalabad, Pakistan²University of Central Punjab, Lahore, Pakistan

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Neurofibromatosis (NF) is a complex genetic disorder that causes tumors. These tumors can develop in nervous system anywhere including brain, spinal cord and nerves. The disease is known to be less common in people with old age. Neurofibromatosis is usually diagnosed in childhood or early adulthood. Neurofibromatosis type 1 (NF1) is also known as peripheral neurofibromatosis or von Recklinghausen disease. NF1 and NF2 are separate disorders resulting from changes in different genes. NF1 gene is located on chromosome 11 and NF2 gene is located on chromosome 22. Only one copy of a mutated or deleted *NF1* gene is required to affect an individual. The affected gene is a tumor suppressor gene that produces the protein neurofibromin. About 50% to 75% of people with NF1 also have learning disabilities. According to previous studies, that the mutant gene can be passed on to future generations. The study is focused on analysis and identification of NF1 mutated genes in diseased patients to explore different genetic variations by using molecular markers.

**GENOTOXICITY ASSESSMENT IN ROHU (*LABEO ROHITA*) FOLLOWING EXPOSURE
TO SUB-LETHAL DOSES OF FIPRONIL****Tehreema Sabat and Irfan Zia Qureshi****Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University,
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Insecticides may enter into the fish body through ingestion or direct contact with the skin, scales or mucus and cause stressful conditions even at low concentrations. The insecticide fipronil is widely used to control various veterinary, residential, and agricultural pests such as beetles, ants, cockroaches, ticks, fleas, weevils and termites. Fipronil blocks GABA receptors in the central nervous system and is highly toxic to aquatic organisms like invertebrates, fishes and also fish-eating birds. Fipronil affects physiological status of proteins and various biochemical parameters. In the present experiments, fish species rohu (*Labeo rohita*) was exposed to different doses of fipronil. Each group contained ten fishes. Group I: control group, Group II: low dose treated with 220 µg/L of fipronil, Group III: high dose treated with 350 µg/L of fipronil. After exposure of 96 h brain, liver and muscle tissues were dissected for assessment of the extent of DNA damage through DNA laddering assay and comet assay. Data from treated groups and control were compared statistically. One way ANOVA and t-test were applied to compare intraspecific and interspecific results, respectively. P<0.05 was considered a significant difference. Treated fish showed disruptive behaviour and impairment in body's balancing system. They became sluggish and motionless. DNA content was found to be significantly increased (p <0.05) in the muscle, brain and liver tissues at high dose of fipronil (350 µg/L). Smear formation was observed in the DNA obtained from muscle, brain and liver tissues of rohu. Comet assay showed significant increase (p <0.05) in olive tail moment and the tail moment of DNA in muscle and liver tissues. No significant difference was noticeable in olive tail moment and the tail moment of DNA in brain tissues. It is concluded that high dose fipronil (350 µg/L) is highly toxic to the fish species. Further studies are required to investigate the molecular mechanisms by which fipronil caused toxic effects in rohu.

3. HUMAN AND ANIMAL DISEASES

A SURVEY ON THE DISEASES ASSOCIATED WITH BLOOD TRANSFUSION IN POPULATION OF KARACHI

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Blood transfusion is a lifesaving technique it may causes adverse effects on population throughout the world. In present study the diseases and infection causes by blood transfusion among the population of Karachi were studied. During the present study Feb 2017 –Dec 2017 from different hospitals of Karachi, 200 patients were examined, in which 178 were infected and 22 were non-infected. The diseases caused by blood transfusion among patients include abnormal bleeding (11%), Diabetes (10%), Hepatitis B (8%), Thyroid disease (7%), Asthma (7%) Hepatitis E (5%), Malaria (5%), TB (5%) Dengue (5%), Kidney disease (4%) HIV (4%), Urine (4%), Hepatitis A (3%) and weight gain (3%). The main cause of blood transfusion diseases was the repeated use of single syringe for the transfusion of different patients.

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS OF *SALMONELLA TYPHI* IN GENERAL POPULATION OF DISTRICT BANNU, KHYBER PAKHTUNKHWA, PAKISTAN

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Typhoid fever is the most serious water and food borne human infection caused by *Salmonella enterica* serovar Typhi and *Salmonella enteric* serovar Paratyphi. Globally the bacterium causes approximately 27 million infections. In various parts of Asia the disease is common especially in children. The present research was designed with the main aim to analyze the rate of infection and clinical manifestations of typhoid fever in general population of district Bannu. In this regards the study was performed from September, 2016 to March, 2017. A total of 4844 individuals, including 1582 male and 3262 female were studied for the presence of possible *Salmonella* bacterium of either sex and with mean age 35.82 ± 15.357 ranged from 1 to 85 years. All the collected samples were screened with widal test, among which 8.14% (8.84% male and 7.78% female) were found positive for typhoid infection and were further processed for confirmation on Typhidot test. Among all (394) the positive widal tested samples, 369 (93.65%) were found to be positive by typhidot test. The test show that 92.15% patients were positive for IgM antibodies and 7.85% patients were positive for both IgM and IgG antibodies and no single case was found to be positive for IgG antibodies. Complete blood count (CBC) was performed for all the widal test positive samples. *Salmonella* infection was also analyzed in various age groups which show that the infection is higher (9.96%) in the age group of 16-30 years and lower (6.18%) in the age group of 31-45 years. Similarly, month wise infection was observed to be higher in the month of October (10.79%) and lower in the month of March (6.83%). Area wise analysis shows that people living in the urban areas were more (71.31%) infected than the rural (28.68%) areas. Blood count analysis shows no relationship with the typhoid infection as the various variables of blood such as Hb, TLC, Neutrophil, Eosinophil, Lymphocytes and Monocytes were normal in majority of the patients. Similarly, the analysis of various risk factors shows that there is close association between the typhoid fever infection and the risk factors such as food, water, contact with typhoid patients and latrine usage. Clinical manifestation analysis were also reported to be the most common in the form of diarrhea, high temperature of more than 40°C, head ache, anorexia, malaise and vomiting. The present study recommended that the government should provide hygienic health care facilities to the people living in poor hygienic areas which will reduce the risk of typhoid fever to a great extent. It is concluded that typhoid fever is endemic in the Bannu district.

DETERMINATION OF THE EFFECT OF LEVAMISOLE TO POTENTIATE VACCINATION RESPONSES AGAINST COCCIDIOSIS IN BROILERS

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Coccidiosis is a parasitic disease of intestinal tract of animals. Its transmission occurs through contact with disease birds and their faeces. The present study was conducted to determine the effect of commercially available Levamisole in response to Vaccination against Coccidiosis in Broilers. Different timing, frequency and concentration of levamisole were used in the each group of three groups (each 50 birds) before coccidiosis vaccination at day 7 of age through intra-peritoneal injection. The birds that have received levamisole (0.25mg/Kg body weight), shown more resistance against coccidiosis and improved weight gain for 4 weeks when compared with vaccinated broiler birds that does not receive levamisole before vaccination. Following challenge, performance parameters were weight gain and liveability. It is concluded that levamisole boost up the vaccination responses in chickens. Therefore, before vaccination the de-worming through levamisole should be done.

PREVALENCE OF DISEASES CAUSES BY URINARY TRACT INFECTION IN FEMALE POPULATION OF KARACHI

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The principle purpose of this study is to gather the information about the causes and symptoms of urinary tract infection (UTI) in female population of Karachi. UTI are the most widespread infection in women. These infection is normally caused by asymptomatic. It influences different part of urinary tract. The infection is frequently caused by *Escherichia coli*. During present study Feb 2017-Dec 2017, total 200 patients from different hospitals of Karachi were examined. Out of 200 patients, 34 (17%) were non infected 166(80%) were infected, in which 166 infected (22%) were infected by dehydration (11%) by vaginal infection (11%) by kidney stone(9%) by poor hygiene (6%) by sexual intercourse (6%) by urine infection (4%) by bacterial infection (4%) by leafy vegetables (2%) by menstrual cycle (2%) by sweating (1%) by constipation (1%) by swelling of bladder and (1%) by inherited. The main causes of UTI are dehydration, due to intake of water. Vaginal infection, kidney stone, poor hygiene condition and intake of coffee and tea in female patients.

RISK FACTORS AND PREVELENCE OF THYROID DISEASE IN FEMALES OF KARACHI

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Prevalence of thyroid disease has become an issue on the epidemic level in Pakistan, prominently in females of Karachi. Karachi has an estimate of about 11.3% of the total population of Pakistan, which includes 5.3% females. Thyroid disease is considered to be most serious disease it causes dysfunction to hormone T3 and T4. The function of thyroid hormone actually depends upon the amount of iodine intake. The amount of iodine intake should not be excessive or very low otherwise it will lead to hypothyroidism or hyperthyroidism. Enlargement of gland causes goiter. The prevalence of thyroid disease greatly exists among the females. Out of 200 patients 164(82%) are infected by thyroid disease due to (19% by pregnancy, 6% after miscarriage, 13% by enlargement of gland or goiter, 7% by loss of iron, 12% by diabetes, 4% by grave's disease, 8.53% by loss of iodine, 6.09% by hormonal misbalance, 6.09% by typhoid, 12.1% by inheritance and 36(18%) are non-infected

with thyroid disease. Thyroid disease is not considered at level of seriousness although this disease is common and diagnosable among the female patients.

**PREVALENCE OF HEPATITIS C VIRUS (HCV) INFECTION IN BANNU
DISTRICT KHYBER PAKHTUNKHWA, PAKISTAN**

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Hepatitis C is one of the most common liver diseases around the world. It cause is hepatitis C virus (HCV) and a significant number of patient's progress towards chronic hepatitis, hepatocellular carcinoma and liver cancer. Worldwide it is estimated that more than 250 million people are affected from HCV. More than 10 million people in Pakistan are infected from hepatitis C. The study was conducted from September, 2016 to August, 2017 in the general population of District Bannu, Khyber Pakhtunkhwa, Pakistan. A total of 1016 individuals of the age group 1 to 75 years were collected from the district Bannu Pakistan. Out of the total 1016 individuals, 611 were males while 405 were female. All the individuals were divided into five age groups and then screened for anti-HCV antibodies with Immunochromatographic (ICT) test. The results show that out of the total 1016 samples, 45 (4.4%) were found positive for anti-HCV antibodies while 971 (95.5%) were found negative for anti-HCV antibodies. The HCV infection was found to be higher in the age groups of 46-60 years (9.7%) and lower in the age group of 1 to 15 years (2.1%). In the age group of 16 to 30 years it was 3.4% and in the age group of 31 to 45 it was 4.4%. The major risk factors that are responsible for the transmission of HCV were dental surgery, piercing/tattooing, blood transfusion, reuse of contaminated blades, reuse of syringes, general surgery, intrafamilial prevalence and drug abuse. Similarly the HCV prevalence was higher in rural areas then the urban areas or city. Beside this the hepatitis C prevalence was higher in married people as compared to unmarried people in District Bannu. Hepatitis C is prevalent in the general population of District Bannu due to illiteracy, lack of proper blood screening facilities, lack of health instrument, no idea about the risk factors of HCV and shortage of scientifically trained health care workers.

**EPIDEMIOLOGY OF DIPHTHERIA IN GENRAL POPULATION OF BANNU AND IDPs
OF NORTH WAZIRISTAN AGENCY (NWA), PAKISTAN**

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Diphtheria is an acute and highly contagious disease of the upper respiratory tract. It is caused by toxigenic, gram positive bacteria *Corynebacterium diphtheriae*, mostly affect children below 10 years. It is one of the major cause of high mortality and morbidity in developing countries. The aim of our study was to investigate mortality rate and to understand the causes of persistence of the disease in Bannu division and IDPs of North Waziristan Agency and to enlighten some strategies to prevent future outbreaks. The study was carried out at DHQ Hospital, KGN Hospital and Women and Children Hospital of Bannu division. Suspected individuals of various age groups and gender with tender throat and pseudo-membrane were part of this study. Laboratory diagnosis was not possible as PCR facilities were unavailable in Bannu division. Our findings suggest that children of 1-10 years of age group were more vulnerable to diphtheria infection (76.5%). The disease was most prevalent during September 2016 to December 2016, highest incidences were reported in November (n=180, 42%) and October (n=91, 21.3%). During the study 12 mortality cases were reported giving the mortality rate of (2.81%). From the findings it is concluded that diphtheria can cause significant mortality and morbidity if not diagnosed and treated early and pre-vaccination is the only way of prevention and control of this dreadful disease.

SEROPREVALENCE OF TOXOPLASMOSIS IN FEMALE BREAST CANCER PATIENTS IN LAHORE

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Toxoplasmosis is a zoonotic infection of humans and animals, caused by the opportunistic protozoan *Toxoplasma gondii*. Infection in pregnant women may lead to abortion, still birth or other serious consequences in new born. Infection in immunocompromised patients can be fatal if not treated. On average, one third of people are chronically infected worldwide. Cancer remains a leading cause of death, responsible for approximately 13% of global deaths. The prevalence of human infection with *Toxoplasma gondii* has been increasing day by day. Thus, the objective of present study was planned to determine the prevalence of anti-*Toxoplasma gondii* antibodies in breast cancer patients by examining the seropositivity and serointensity rate of *Toxoplasma gondii*. By using ELISA kit the seroprevalence of toxoplasmosis was analyzed. To find out the risk factors, all the information was collected with the help of questionnaire. 130 blood samples were collected from Sir Ganga Ram hospital Lahore out of which 90 samples were case and 40 samples were control. In a 90 samples of case 42 were seropositive and 48 were seronegative. In a control group out of 40 samples of case 14 were seropositive and 26 were seronegative. Among breast cancer and non breast cancer female's prevalence rate was 46.6% and 35% respectively Overall prevalence in breast cancer female population in Lahore was found 46%. To reduce the infection rate in a local population of Lahore, health education and public awareness is needed.

ESTIMATION OF THE BIOCHEMICAL AND OXIDATIVE STRESS STATUS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) PATIENTS FROM LAHORE-PAKISTAN

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Chronic obstructive pulmonary disease (COPD) is the sluggish devastation of airways and ultimately hammering of lung function. COPD is a mixture of chronic bronchitis and emphysema. Worldwide 11%– 21% of the inhabitant elder than 40 years (predictable 80 million) suffering from COPD. Reported menace factors for COPD consist of genetic tendency, tobacco smoking, professional contact to dusts and vapors, inside (use of bio-mass petroleum, particularly in rising countries) and outside air pollutants, elderly, infections, asthma and low socio-economic status. 5.0 ml blood sample of 60 diagnosed COPD patients and 50 samples of healthy individuals were taken from vein in gel clotted vial from the Jinnah Hospital and Gulab Devi Chest Hospital Lahore. Blood was auxiliary processed for the evaluation of Catalase (CAT), Reduce Glutathione (GSH), Malondialdehyde (MDA), Superoxide Dismutase, Nitric oxide, estimation of different micronutrients such as Vitamin A, C and E and Electrolytes concentration (Na^+ and K^+) through flame photometer. MDA was found in COPD diagnosed patients (7.96 ± 0.57) and in healthy individuals (1.28 ± 0.23). The concentration of vitamin C in the people suffering from COPD was (3.19 ± 0.10) and the control group had the value of vitamin C as (6.25 ± 1.03). Nitric Oxide (NO) was in healthy persons (15.18 ± 2.03) while in those persons affected with COPD, its value was (41.03 ± 0.61). CAT value for healthy controls was (4.23 ± 1.05) while it was found as (3.01 ± 0.000) in COPD diagnosed patients. The p-value = $0.000 < 0.05$ was found in all parameters and it showed that p-value was significant statistically. The results of present study showed that the level of MDA is highly increased and so it is clear that the patients with more severity of COPD have active process of lipid peroxidation. Overall results showed that the decreased levels of vitamins, GSH and enzymatic antioxidants as well as increased levels of Lipid peroxidation and electrolyte balance (Na^+ and K^+) may be involved to the development of COPD. Further studies are needed to analyze the pathophysiological procedures in the COPD.

IMPORTANCE OF ZOONOTIC BOVINE TUBERCULOSIS AND CONTROL STRATEGIES

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Bovine Tuberculosis is a chronic infectious zoonotic disease of domestic animals, wide range of wild animals and humans. *Mycobacterium bovis* is the main causative agent of Bovine tuberculosis (zoonotic TB) which is an acid fast staining bacterium due to waxy substance (mycolic acid) present in its bacterial cell wall. The bacteria have the capability to transmit by both aerogenous and enterogenous routes. Bovine tuberculosis (BTB) always remains a major public health concern as emerging and an important extrapulmonary human tuberculosis. It is considered as a remarkable health concern of animals having great economic importance and a major contribution in the global disease burden specially in the developing countries. It results in important economic losses and trade barriers with a major impact on the livelihoods of poor and marginalized communities with no known geographical boundaries. Globally, most cases of zoonotic TB are caused by *M. bovis*, and cattle are the major reservoir. *M. bovis* causes a relatively small proportion, less than 2% of the total number of cases of TB disease leads to accounts for less than 230 TB cases per year in the United States. It is associated with the progressive weakness, disorder of respiratory systems primarily in the lungs which then with the passage of time pass to other organs. The transmission of *Mycobacterium bovis* from animal to animal occur through colostrums/milk to calves, by the ingestion of the infected flies, by the dropping of birds, aerosols, contact with one and the other animals. It is transmitted from animals to humans through unpasteurized contaminated milk and its by-products, sputum, urine, visceral organs, nasal discharges or aerosols and through close contact with infected animals. Transmission of *M. bovis* can occur between the animals, from animals to humans and also through humans to animals, but rare from human to human. Tuberculinization test, single intradermal test and comparative intradermal test, is the valuable delayed-type hyper-sensitivity test used for diagnosing TB in live animal, and used in TB eradication and for international trade. Vaccination of calves with attenuated bovine-strain of tuberculosis bacterium, known as Bacillus of Calmette and Guérin (BCG) and testing and culling are important measure in BTB control and prevention worldwide. It is important to pasteurize milk before human consumption to reduce public health risk. The association of bovine tuberculosis with unhygienic food/ malnutrition and poverty has long been recognized and the need to address these basic issues are as crucial as specific measures against the disease itself.

INCIDENCE OF KERATOCONUS IN LAHORE POPULATION AND ITS ASSOCIATION WITH NUMBER OF DIFFERENT OCULAR AND SYSTEMIC DISORDERS

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To determine the prevalence of Keratoconus in male and female of various age group in Lahore and to identify the severity of disease in Keratoconus patients. A cross-sectional, analytical study was performed between July 2016 to August 2017 on Keratoconus patients visiting Layton Rahamtullah Benevolent Trust Hospital and Sir Ganga Ram Hospital during their ophthalmic consultation. Clinical evolution for Keratoconus was performed by Visual Acuity, Snellen chart, Ophthalmoscopy, Orbscan corneal topography and Slit lamp biomicroscopy with the help of ophthalmologists. One hundred and ten patients of Keratoconus were studied in which females affected more (n=62) as compare to males (n=48) including patients with family history of Keratoconus (n=21) as well as patients have history of eye rubbing (n=61). Both unilateral (n=52) and bilateral conditions (n=58) were observed. Prevalence of normal, moderate and high vision loss were also observed with 10%, 29.09% and 60.61% respectively. Signs of Munson's (n=68), Rizutti's (n=30), Vogt's striae (n=26), Atopic syndrome (n=19) patients, Leber's Congenital Amaurosis (n=6) and Fleischer (n=23) were also present in Keratoconus patients. 29 cases of Keratoconus had mild<45D k-readings, 47 cases had moderate 45>52D k-readings and 34 cases had severe>62D k-readings. Females are more affected as compare to male with Keratoconus. Both unilateral and bilateral condition also present with association of different types of diseases.

PHYTOCHEMICAL SCREENING OF *IRELINE HERBSTII* (CHICKEN GIZZARD PLANT) AND ITS ANTIVIRAL POTENTIAL AGAINST NEWCASTLE DISEASE VIRUS

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Newcastle disease is among the most devastating viral disease of poultry and the causative agent of this disease is Newcastle disease virus. Medicinal plants have been used in the treatment of diseases and infection, including viral infections. The ability to synthesize compounds by secondary metabolism possessing antiviral potential makes plants an invaluable source of pharmaceutical and therapeutic products. *Iresine herbstii* commonly known as chicken gizzard plant was used for their antiviral potential against the Newcastle disease virus. The shoot part of this plant was placed for *in ovo* trial. Qualitative phytochemical analysis for all four tested solvents of *Iresine herbstii* was performed. Results of phytochemical screening reveal the presence of all tested phytochemicals in ethanolic extract, acetone extract did not have phenols and tannins, dichloromethane showed the absence of tannins and terpenoids and petroleum ether extract showed absence of all phytochemical substance except alkaloids, flavonoids, glycoside & phenols. To check the antiviral potential of plant, different prepared treatments of plant extract and live virus were inoculated in 9 days old embryonated chicken eggs. Embryo survival rate was observed daily up to 72 hours. Results exposed that all plant extracts produce antiviral activity against NDV *in ovo* according to their potential and phytochemical profile. Highest survival rate was observed in ethanolic extract at 400µg/mL and acetonic extract at 300µg/mL as it control the NDV activity completely, evidence by absence of embryo death and HA titre. Dichloromethane and petroleum ether could not inhibit the virus completely. 600µg/mL concentration was proved as toxic in all extracts except petroleum ether extract which showed dose dependent pattern.

PREVALENCE OF EQUINE DERMATITIS AND ASSOCIATED RISK FACTORS IN SELECTED DISTRICTS IN KELANTAN, MALAYSIA

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Equine dermatitis causes major discomfort to the animal and in extreme cases, may lead to equine mutilating itself in an effort to seek relief from itching (pruritus) by scratching or biting the inflamed skin. Equine dermatitis affects the overall health and sheen of the animal's coat and can turn the skin red and flaky. However, no study about equine dermatitis had been conducted in Kelantan, Malaysia. So, this study aims to find the prevalence of equine dermatitis in five district of Kelantan, Malaysia, as well as investigate the associated risk factors contributing to disease occurrence. Eighty horses were sampled randomly from five districts and diagnostic tests carried out were acetate tape preparation, skin scraping, fungal culture and microscopic examination. The results of the study reveals that 51.2% prevalence of equine dermatitis in Kelantan. Prevalence decreases with age, 62.5% in those < 5 years, 48.1% in those 6-10 years and 44.8 % in horses > 11 years. According to breed, 38% of prevalence in crossbred, 59% in Thoroughbred and 45.7% in local Padi. Female shows high prevalence (54%) compared to male (29%). Risk factors associated with the disease are less frequent stable cleaning, lower frequency of grooming and sharing of grooming kit among the horses. Among causative organisms of dermatitis, bacteria (*Staphylococcus* spp., *Dermatophilus congolensis*, *Streptococcus* spp. and *Norcadia* spp.) constitute 55%, fungal infection (*Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Microsporum gypseum*) entails 36%, traumatic injury plays 7% and lice infestation is 2%. The present may provide the basis for future studies on equine dermatitis using more samples and sampling area and the risk factor associates with the problem.

ASSESSMENT OF LACTOSE DEHYDROGENASE IN LEUKEMIA PATIENTS VISITING CHILDREN HOSPITAL, LAHORE

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The present project was designed to study the assessment of Lactose dehydrogenase (LDH) in leukemia patients. It was done at Children Hospital, Lahore. For this purpose 150 blood samples of leukemia patients were collected from April 2014- July 2014 and 30 blood samples of normal persons were also collected and the level of LDH was checked by a method using Auto Analyzer (Roche/ Hitachi 902 Analyzer). It was noted that out of 150 samples taken, 89 were taken from male and 61 were taken from females who had leukemia. The percentage of male and female was 59.3% and 40.7% respectively. The prevalence of type of leukemia was also noted. Out of 150 patients, 136 had Acute Lymphoblastic Leukemia (ALL) and 14 had Acute Myeloid Leukemia (AML). The percentage for ALL was 90.7% and for AML was 9.3%. Age and economic status of leukemia were also noted. Most of patients belong to low economic status and lie in the age group of 1-5 years. The LDH level was also noted in subject and control groups. The subject group had greater LDH level (731 ± 240.0) than control group (200.3 ± 53.7). The LDH level was also observed in between AML patients and control, the level is higher in AML (1132.0 ± 327.7) than control. The LDH level was also noted in between ALL and control, the value is greater in ALL (712.8 ± 188.5) than normal. The LDH level also noted in between AML and ALL, the level is greater in AML (1162.3 ± 58.7) as compared to ALL (702.5 ± 192.0). And all the results were highly significant.

SEROPREVALENCE AND RISK FACTORS OF TOXOPLASMOSIS IN PREGNANT WOMEN OF DISTRICT SWABI

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Toxoplasmosis is parasitic infection that causes severe abnormalities in newborn babies when transmitted from mothers. The present study was conducted to find the seroprevalence and risk factors of toxoplasmosis among the pregnant women of selected samples taken from District Headquarter Hospital Swabi. Furthermore, the selected samples were scrutinized for toxoplasmosis in relation with gestation period, age factor, total number of pregnancies women carried, socioeconomic factors and the source of contamination through which women can be infected. Blood samples were collected from pregnant women of maternity ward at District Head Quarter Hospital, Swabi. The sera sample was examined for the presence of IgG/IgM anti-Toxo antibodies. Overall prevalence of toxoplasmosis was reported to be 12% among which 05 cases were positive for only IgG and remaining 07 were positive both for IgG and IgM. Regarding gestation period, females at the third trimester of pregnancy were at greater risk and about 20% of cases were screened positive. Similarly in relation to age factor, women at the age of 31-36 showed high prevalence (25%) for toxoplasmosis. Examining toxoplasmosis in relation to number of pregnancies women carried, high percentage was recorded in women with their first pregnancy (3.96%). The findings also linked the infection with socioeconomic factors i.e. (36.36%) of cases were reported in pregnant women belonging to lower class. Similarly, in reporting results for toxoplasmosis due to contaminated environment, 30% of the cases of toxoplasmosis were mainly caused by the consumption of raw/improperly cooked meat. Other enormous causes included the interaction of women with cats and contaminated soil i.e. 27% each. Proper screening of women during pregnancy and awareness about the infection by health departments may contribute towards the prevention of congenital Toxoplasmosis.

PREVALENCE OF MALARIAL PARASITES (*PLASMODIUM VIVAX* AND *PLASMODIUM FALCIPARUM*) IN BLOOD OF HUMAN PREGNANT FEMALE POPULATION OF LAHORE

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Present study was designed to find out the prevalence of malarial parasite (*P.vivax* and *P. falciparum*) in pregnant female population of Lahore. Blood samples were collected from different hospitals in Lahore and detecting malarial parasite microscopically. Out of all 300 samples 24(8%) showed positive seroprevalence for *P.falciparum* and *P.vivax*. while 276 (92%) were negative for malaria. Trophozoites and gametocytes were the erythrocytic stages of *P. vivax* and *P. falciparum* observed during the study. Prevalence was high in (18-28) years age group as 18(75%). It was observed that malaria caused low blood pressure in most of the patients as in 15(62.5%). Seroprevalence was high in low educational areas and in rural areas of Lahore as 15(62.5 %) and in the areas where drainage and sewerage system was poor as 14(58.3%). As most of the seropositive belonged to middle class or lower class families, they have fewer resources for malaria prevention such as insecticide treatment (ITNs) and availability of mosquito repellent lotion. Seroprevalence was high in multigravida female, and in patients that are from 2nd trimester as 10(41.6%). It was observed that most of the patients did not know the mosquito bite and danger and hence risk of being bitten. Pregnant women are particularly vulnerable to malaria because pregnancy reduces a women's immunity to malaria, making her more susceptible to malaria infection and increasing the risk of illness, severe anaemia and death.

PREVALENCE OF DIABETES MELLITUS IN PATIENTS VISITING BACHA KHAN MEDICAL COMPLEX, SHAHMANSOOR, DISTRICT SWABI, KHYBER PAKHTUN KHTWA, PAKISTAN

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Diabetes mellitus is a chronic disease associated with high rate of morbidity and mortality in developing world. Diabetes mellitus occur due to relative or absolute deficiency of insulin hormone released from β cells of islets of Langerhans of pancreas. The most common types are T1DM and T2DM but such other types also found. The study was undertaken to determine prevalence of diabetes mellitus among the attendance in Bacha Khan Medical Complex Shahmansoor Swabi. A cross sectional survey was conducted from the month of October 2016 to may 2017. The whole study consists of total 189 patients that visiting Bacha Khan Medical Complex Shahmansoor, Swabi, in which females were 130 and males were 59. Gender wise distribution of patients show high prevalence in females that were 130 than males that were 59. Also divided the number of patients into age wise groups in which high prevalence found in the age of 61-70 years patients that were 48 patients. Monthly wise distribution show high prevalence in the month of March that was 38 patients. And on the basis of types of diabetes high prevalence found on other types of diabetes than T1DM and T2DM that affect 142 patients.

PREVALENCE OF DIARRHEA IN PATIENTS VISITING DHQ HOSPITAL ADAMZAI, DISTRICT NOWSHERA, KHYBER PAKHTUNKHUWA, PAKISTAN

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Diarrhea is related to infection in gastro intestinal tract which can be caused by a variety of bacterial viral and parasitic organisms. People of all age get diarrhea but it is more common in children's below 5 ages. Other causes of diarrhea is poor hygiene contaminated water and food. Globally there is 2 billion cases of diarrheal disease every year. Nearly one in five child deaths, about 1.5 million each year, is due to diarrhea. In Pakistan

30% of people is suffer in which 25% is controlled. The purpose of this study is to identify the disease its damages and its prevalence among the attendance in medical wards of DHQ hospital of Adamzai District Nowshera. A cross sectional survey was conducted from the month of March 2017 to May 2017. The whole study consists of 215 patients admitted in medical wards of DHQ hospital Adamzai in which children's were 133 and adults were 83. Gender wise patients is dispersed it show high frequency in males that were 120 than females that were 95. Also divide the number of patients into age wise groups in which high prevalence found in the age of 1 to 10 years that were 75 patients and on the basis of month wise distribution show high prevalence found in May that were 90 patients.

A STUDY OF CANINE PARVOVIRUS INFECTION AND THERAPEUTIC RESPONSE OF DIFFERENT IMMUNE BOOSTERS

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Humans and domesticated dogs relation is as old as the human civilization itself is. This special bond has amazingly survived the long and dusty distance of dozen of centuries, from the life of pre historic man to the semi-robotic life of today's men. There are many diseases of dogs, from which some are very lethal and dangerous for dog's life. These diseases require huge amount of money and special treatment protocols for the recovery of animal health. One of such most important disease is Canine parvovirus (CPV). Canine parvovirus the most deadly disease of dogs is manifested in dogs by inflammation of the intestine and loose motions. If the blood starts coming in the diarrhea with a typical foul smell then it is cent per cent positive case of parvovirus. CPV is taken as a new disease as it was first identified in the ninety seventies. And in a matter of few year it was the commonly found virus of the world. It is also thought that CPV has its origin from feline enteritis virus with a difference of only 2 or three amino acids. The main area of difference is the VP2 region. This virus has some similarity with the mink enteritis virus. Some evolutionary scientist claims that the CPV might be a product of some wild virus. It is also confirmed by the fact that a subtype of CPV causes disease in even cats. However the original CPV is specific in its host range effecting only the canines. In present time the newly emerged subtypes of CPV are causing most of the disease around the globe. These two viruses has assumed the role of their originator very effectively. Now most of the diseases are caused by these variants. And this is the reason of vaccination and diagnostic failure as the new stains are not detected by and included in the vaccine formation process. In addition to this this family has expanded with the entry of third variants that was identified in the Italy. CPV is a non-enveloped DNA virus which belongs to family Parvovirinae. The meaning of this virus is small while its size is so minute that it is only about 20 nm size. . The importance of CPV is elaborated the rate of mortality that is above ninety percent (Decaro *et al.*, 2014), however the immunity of maternal side gives a protective coverage in case of young dogs. In Pakistan, disease has been reported among dogs presented to private veterinary clinics (Manzoor and Jamil, 2013; Umar *et al.*, 2015). Nevertheless, CPV investigation were based on haemagglutination and inhibition by polyvalent serum which are considered less efficient in term of diagnostic sensitivity and specificity and surely would not predict true burden of infection among susceptible population. Thus, the diagnosis of CPV should be based on demonstration of virus in the feces using monoclonal antibodies rather than clinical picture and other less sensitive assays.

PREVALENCE OF MALARIA IN SWABI TEHSIL AND LAHOR TEHSIL OF DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Malaria is a parasitic disease, which is most commonly transmitted by an infected female *Anopheles* mosquito. It is usually found in tropical and subtropical climates of the world. Five species of *Plasmodium* can infect humans. Which are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Common symptoms

of malaria include irregular fever, chills, headache, vomiting, Diarrhea and weakness. Malaria is typically diagnosed by the microscopic examination of blood using blood films. The several rapid diagnostic kits also exist for malaria diagnosis. Pre-erythrocyte vaccine, blood stages vaccine, Anti-mosquito's stage vaccines and transmission-blocking vaccines (TBV) are designed to prevent malaria transmission. Malaria is transmitted by blood transfusion, sharing syringes and organ transplantation. It can be prevented by using insecticide treated nets, insecticides spray and also using protecting clothes. The present study was conducted to investigate the prevalence of malaria in males and females during the month of October to April (2016-2017) in Swabi & lahor tehils of district Swabi, Khyber Pakhtunkhwa, Pakistan. A total of 600 patients were diagnosed for malaria in different health care centers of Swabi & lahor tehils of district Swabi.

COMPARATIVE OCCURRENCE OF HEPATITIS B IN LOCAL POPULATION OF DISTRICT SWABI

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Pakistan carries one of the world's highest burdens of chronic hepatitis and mortality due to liver failure and hepatocellular carcinoma. In Pakistan about 10-12 million of the people are suffering from hepatitis. It is estimated that there are about 400 million of carriers of HBV all over the world. This study was conducted in the Bacha Khan Medical Complex Swabi. A total of 3,977 patients, belonging to different localities were screened for HBsAg by using ELISA (enzyme linked immunosorbent assay) and ICT (Immuno chromatographic technique). Out of 3,977 cases, 81(2.03%) were positive for HBV. The common symptom noted among most of the patients was loss of appetite (65%). Co-infection was found only in 6 patients. The common risk factors associated with HBV was found such that history of dental treatment was 70%, treatment from unqualified doctor 49%, history of blood transfusion 43%, history of minor/major surgery 40%, HBV positive patients in family 35%, shaving from street barbers 32%, diabetes 16%, tattooing 11%, HCV co-infection 7.41%, sharing of drug injecting equipment 1.2%, HIV and T.B co-infection was 0%. The highest %age was noted in the area of Swabi (0.58%). The +ive patients were mostly young adults of age range from 21 to 40 years.

PREVALENCE OF TUBERCULOSIS IN DISTRICT SWABI, KPK, PAKISTAN

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Although Swabi has proficient rapid economic growth over the decades, significant health and nutrition problems remain unfortunately because little work has been done to track basic diseases such as anemia; exact prevalence of these health problems is often unknown. The goal of this research is to assess the prevalence of Tuberculosis in District Swabi. Tuberculosis (TB) is as ancient disease and lead cause of death worldwide. Recurrence and involvement of TB with HIV have made TB a nagglobal hazard again. Tuberculosis is more widespread in developing countries. The present study was carried out in Swabi in seven months (November 2016 to May 2017) to check the prevalence of tuberculosis in Swabi as well as to observe its type that are (Smear positive and Smear Negative) in past two years. The results of our present study revealed that a total of 450 new cases IN 2016 have been registered in DHQ center Swabi. From our results it has been observed that in Swabi from November 2016 a total of 450 patients Tuberculosis reports came to DHQ center Swabi for the Examination of Tuberculosis. A total of 288 peoples were smearing positive for pulmonary Tuberculosis and 100 peoples has smear negative. Out of these smear positive 135 were Female and 153 were male's patients. There are 100 patient having smear negative tuberculosis Out of these smear negative 64 were males and 36 were female's patients. Out of 450 patients 62 people has been Relief from tuberculosis in which 27 were male adults and 35 were children of both genders (male and female). In 2017 total patients were 127 in which smear positive 121, relief 06. Out of smear positive 46 males and females 35(adults) and children 40 respectively. From our study it has been concluded that TB is still a major health problem in Swabi. Therefore awareness

among people should be increase by the government as well as health department to decrease the ratio of tuberculosis in people of Swabi and globally as well.

PREVALENCE OF ANEMIA IN PRIMARY, MIDDLE AND SECONDARY SCHOOLS GOING CHILDREN IN TEHSIL CHAMLA, DISTRICT BUNER, KHYBER PAKHTUNKHWA.

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Anemia is a deficiency in the size or number of red blood cells (RBCs) or in the amount of hemoglobin they contain. It is a blood disorder in which the hemoglobin (Hb) concentration is less than the normal Hb concentration for age, gender, physiological condition and altitude. Anemia is a serious problem among Pakistani children. The common causes of anemia include parasitic infestations such as malaria and hookworm; Infections like HIV and hemoglobinopathies. This study, was done to keenly observe the level of hemoglobin in school going children in Tehsil Chamla, District Buner, KPK. Data was collected from primary, middle and secondary schools of Tehsil Chamla, District Buner. On primary and middle level one school was selected from each union council. While on Secondary level selected one school from union council Nawagai, one from council Makhraanai and two schools were selected from union council Kawga. Total children's of Primary Schools were 410 and sample size 51. Similarly Middle Schools children's were 410 and sample size were 27 while Secondary Schools children's were 377 and sample size were 24. In Primary Schools among (51) students (14) were anemic equal to 27%. Similarly in Middle Schools, among (27) students (7) were anemic equal to 25%. In Secondary School among (24) students (5) were anemic equal to 20%. Maximum anemic students were observed in primary schools followed by Middle and secondary Schools of Tehsil Chamla. The percentage of anemia in children was higher from 1st to 8th class (52%) as compare to the children of 9th to 12th class (20%). It clearly shows that balanced diet has a great impact on children's health. It is hence concluded that higher rate of anemia is observed where there is lower income of family and lesser health facilities and vice versa.

PREVALENCE OF ANEMIA IN PRIMARY AND MIDDLE SCHOOL OF SIL DARGAI DISTRICT MALAKAND KPK, PAKISTAN

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The present study was conducted to contemplate the hemoglobin level in school going children of Tehsil Dargai, District Malakand, Khyber Pakhtunkhwa. The data was collected from primary and middle school of Tehsil Bargain the primary school were govt primary school Koper govt primary school Qadar Kalai, govt primary school Hijab Kalai, middle school Sina Public School Stanadaro Kalai, Standard Education Wazir Abad Bargain, and Malakand Children Academy. From each school 20 students were selected for collecting of the blood and the analyzed hemoglobin concentration. The blood sample were collected in EDTA (ethylene diamine tetra acetic acid) from different school by self. Hemoglobin level was determined by bio chemistry analyzer model Techno 786 having two reagent, cell pack and stromatolyser wwt 500 ml. Total sample from primary school were 60 and middle school sample were 40. In primary school among 60 student 15 were anemic equal to 25% in middle school among 40 student 7 were anemic equal to 17%. Maximum anemic student were observed in primary schools followed by middle schools of Tehsil Bargain. The most important cause of anemic children was observed in Tehsil Dargai these are the rural region of District Malakand it means the anemia was more prevalent in rural region than in urban area. During interview it was observed that anemic children were weak the percentage of anemia in children of lower class families was higher 25% as compare to children of upper class families 17%. It clearly shows that balanced diet has a great impact on children health. It is hence concluded that higher rate of anemia is observed where there is lower income of family and lesser health facilities and vice versa.

PREVELANCE OF HEPATITIS C VIRUS IN PATIENTS VISITING DHQ HOSPITAL, SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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The beginning of hepatitis C virus (HCV) can be conceptualized at numerous stages. HCV very common in Swabi due to poor hygienic environmental condition and due to low socio-economic values initially, backgrounds strength mention to its theatrical binge during the twentieth century. As plasma born. Medicinal actions, immunization, blood transfusion and extra newly vaccinating drug use. Hepatitis is deadly viral infection caused by hepatitis c virus it is the major cause of chronic liver diseases. Cirrhosis, hepatic cellular and development of hepatocellular carcinoma. it accounted for 23% of viral acute disease and of chronic liver diseases the present study were conducted in Swabi DHQ hospital for last two years 2015 to 2016 during (2017).The data collected in the of \months of Jan and Feb. (2017).Out of 4000 patients 1500 were infected by HCV and 2500 were non HCV. in the month of Jan (2017) 880 patients were screened out of 1500 while 1000 were non HCV cases. In month of Feb (2017) 620 patients were screened out of 1500 while 1500 were non HCV cases. Patients belonging to various areas of Swabi were tested for anti-HCV.by using immunochromatographic technique and confirm conducted in the laboratory of district head quarter hospital in Swabi.

ESTIMATION AND CORRELATION OF MALONDIALDEHYDE (MDA) AND BIOCHEMICAL RESPONSE IN PATIENTS OF PROSTATE CANCER

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Cancer is a prominent health risk for men all over the world. Prostate cancer is also general class of cancer in men and the second major cause of cancer death in men after lung cancer .The prostate is a male gland situated beneath the bladder that starts to develop earlier birth because of androgens. The role of the prostate is to reserve an alkaline fluid, which compose almost one-third of the volume of semen. 5.0 ml blood sample of 30 diagnosed Prostate cancer and 25ae healthy individuals will be taken from vein in clotted gel vials from oncology department of Mayo hospital and Jinnah Hospital. The spectrophotometric reading of samples portrays that MDA level in prostate cancer patients is remarkably inflated than normal person (8.42 ± 0.53).Whereas there level in healthy individual is extremely low ($2.25 \pm .24$). The value of GSH demonstrates that in cancer patients the level of GSH reduced (2.12 ± 0.02) as compared to normal individual (5.32 ± 0.15). The CAT level is moderately decreased in patients than normal person (2.74 ± 0.19). The value of healthy individual is high (4.11 ± 1.05).Results parade the amount of SOD that is slackening in prostate cancer patients (1.14 ± 0.21) though it is high in normal people (3.12 ± 0.25).PSA values elevated in effected person (11.31 ± 18.38) than normal values (7.45 ± 1.51).This data shows that data is statistically significant (0.002). The score prevailed from prostate cancer patients greater (10.30 ± 0.19) than normal person (2.01 ± 0.37). This indicates that data is statistically significant ($P=0.000$). Reactive oxygen species play an important role in carcinogenesis. It has been reported that changes in MDA level and reduced glutathione were associated with the pathogenesis of breast cancer whereas increase in MDA level but decrease in GSH activity in blood was linked to metastasis.

VECTOR BORNE DISEASES ARE RISING IN KARACHI

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The Vector borne diseases constitute to around 17% of all the human infectious disease. VBDs are transmitted through the bite of infected insects (arthropod), such as mosquitoes, sand flies, bugs and ticks etc.

They are causing disaster in the tropical and sub-tropical regions of the globe. These vectors are responsible to carry infected pathogens such as virus, bacteria and protozoa etc from one host to another. The arthropods are ectothermic i-e cold blooded so, they are very sensitive to climatic changes, therefore the climate is one of the important factors among all. The climatic condition directly influenced on the vectors their activities, habitat, distribution, density, reproduction, intensity, maintenance, rate of pathogen development and transmission. The low and high extremes temperature may also affect on the human and pathogen relationships. VBDs like chikungunya, dengue, malaria and zika are prevalent to many countries, these diseases not only involved in mortalities of man but some of them cause high morbidity rate. their pathogens can be transferred from symptomatic human host to a new uninfected human host by the insect vectors. In recent years, several important vector borne pathogens like chikungunya and dengue viruses have been introduced into Karachi-Pakistan. Chikungunya is a tropical VBDs caused by *alphavirus* belongs to the family *Togaviridae* transmitted by *Aedes aegypti* or *Aedes albopictus* caused acute illness which is characterized by high fever, rashes and severe body and joints pain. The pain continues for a months or a year. The incubation period in human is around 4 days whereas infective stage on an average lasts for 7 days with a range from 4 to 11 days. The outbreak of chikungunya has been reported in December, 2016. Dengue is the important arboviral disease and leading cause of death and sickness of man transmitted by *Aedes aegypti* or *Aedes albopictus*, symptoms included high fever, severe headache, severe pain behind the eyes associated symptoms are joint pain and bone pain with rashes and mild bleeding. Primarily symptoms appear 3-15 days after the mosquitoes bite. Dengue hemorrhagic fever is a more serious form of dengue infection. The first outbreak of dengue was seen during 1994 to 1995 in Karachi and cases are continuous reported from the city. Malaria is a life-threatening disease transmitted through the bite of an infected female *Anopheles* mosquito that carries the different species of *Plasmodium* parasite. Symptoms are typically included fever, tiredness, vomiting and headache. Symptoms usually begin 10-15 days after mosquito bitten. The present investigation was done in Karachi during December 2016 to December 2017 among 198 selected symptomatic patients. Patients were referred to the hospital for diagnostic tests. In Hospital, Serological diagnosis was done by testing serum or plasma to detect virus. The result shows that out of 198 patients, only 71 patients were found to be infected with vector borne diseases. According to diagnostic report 38 patients having chikungunya virus, 21 were reported dengue virus and 12 were malarial patients. It is concluded that chikungunya viral disease was initiate to be the major disease among VBDs in Karachi during the current year whereas dengue affected less people as compare to chikungunya as but more than malaria. The study revealed that VBDs. are continue rising in Karachi, it is also indicating that the rate of vectors *Aedes aegypti* or *Aedes albopictus* is going to higher than other mosquitoes. This is very alarming not only for citizen of Karachi but also for Provincial and Federal Government. Eradication program should be started for mosquitoes control, which help to reduces infection of VBDs and public should aware to reduce exposure to vectors and spray around and inside the home, to use mosquitoes repellent, bed net, window screening, use of full sleeves and socks etc.

INCIDENCE OF HEPATITIS C AND HIV AMONG ADDICTS OF SOUTHERN PUNJAB

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Blood transmitted diseases (hepatitis C, HIV) are major public health problems. Drug users, especially injecting drug users (IDU), are by nature of their illness, a risk population for these diseases. The aim of the present study was to estimate the incidence of Hepatitis C and HIV among addicts of southern Punjab. A total of 300 venous blood samples were collected from addicts visiting police line hospital Bahawalpur for rehabilitation. Blood samples were analyzed by immunoassays to detect HIV and HCV antibodies. For HIV, Western blot or immunofluorescence assay was used for confirmatory testing. Final HCV antibody prevalence was estimated using RDS adjustments. It was seen that 76% addicts were having hepatitis C and 9% addicts were infected with HIV. The high incidence of HCV indicates that there is an urgent need to expand HCV counseling and testing for addicts in different areas of Punjab, and to implement interventions that will decrease HCV associated injection risk behaviors in order to prevent a possible surge in the incidence of HIV infection in this population.

PREVALENCE OF SKIN DISEASES WITH RESPECT TO GENDER IN DISTRICT SARGODHA, PAKISTAN

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The aim of this cross-sectional study was to investigate the prevalence of skin diseases with respect of gender in Sargodha. For this purpose clinical survey was conducted from September, 2015 to July, 2016 at DHQ Sargodha. A total of 876 (female 527 and male 349) patients were studied. Infectious cases 66.9% and non-infectious cases 33.1% were observed. Scabies were 41.1%, Psoriasis 16.7%, fungal infection 11.9% and eczema 9.5% were observed as dominant diseases. Patients having poor hygienic conditions were 52.5%, moderate 23.3% and with good hygienic conditions were 11.1%. With respect to gender females were exposed to more skin diseases as compared to males due to poor hygienic conditions, over crowdedness, usage of shared clothes and contact with children.

RISK OF CARDIOVASCULAR DISEASE AND EXPOSURE OF ORGANIC POLLUTANTS

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In developed states cardiovascular illness is the frequent source of mature deaths and globally death rates are increasing day by day. The aim of this study was to determine association between serum lipid profile and risk of cardiovascular disease with persistent organic pollutants. Gas chromatography technique is used to measure the intensity of PCB congeners and organochlorine pesticides residue mg/kg in serum samples. Logistic regression model (Binary logistic regression analysis) was used to conclude the dependent variables (lipid profile) association with independent covariates such as pesticides. In the current study, subjects were divided into three categories according to age groups such as 23-32 younger, 33-42 middle and 43-53 older. Our result represent 78%, 73% and 66% elevated level of TC in younger, middle and older age groups respectively than the standard level. TG level are higher in younger 91% older 89% and 78% in middle age group than normal values. LDL-C was increased in equally both in middle and older age groups 78% and 50% in younger age group than standard values. In addition HDL-C is correspondingly decreased (counter affect) in all age groups. The average values of HDL-C are 42% in 43-53 age group, 41% in 23-32 age group and 26% in 33-42 age group shows high risk of CVD. The mean concentration of pesticides level was calculated higher in middle age group as 0.7390 (mg/kg) than other age groups found as 0.6755 (mg/kg) in older and 0.3416 (mg/kg) in younger age group. The Hosmer and Lemeshow (Chi square) goodness of fit for TC model is 7.910 with 0.442 significance, for TG is 6.588 with 0.582 significance, similarly goodness of fit for LDL-C is 6.134 with 0.632 significance and HDL-C is 4.390 with 0.820 significance. So experimental investigation represents that polychlorinated biphenyl and organochlorine pesticides exposure was strongly related with high lipid level and this change in lipid level are the cause of cardiovascular diseases.

PREVALENCE, EPIDEMIOLOGY AND RISK FACTORS ASSESSMENT OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) IN THE FAMILIES OF AIDS PATIENTS IN AJK

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Acquired Immuno-deficiency syndrome is an infection caused by Human Immunodeficiency virus

(HIV). HIV attacks and destroy the cells of Immune system. This is the leading epidemic of the world. In present study, we find out the prevalence, risk factors and the spread of HIV among the family members of AIDS patients. The family members of AIDS patients were screened by using Immuno-Chromatographic Technique (ICT) kits. The samples appeared positive in ICT were later confirmed by Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). The possible risk factors were analyzed through questionnaire. During study, the family members of 134 AIDS patients including 85 males and 49 females were screened for the presence of HIV. 177(59 percent) individuals were found positive for HIV. Seven out of 25 HIV positive children were found to have developed AIDS. A total of 75 females were positive for HIV and 49 of them had developed AIDS symptoms and all were infected by their male partners. The epidemiological study reveals that among the male patients, 56 (65.88 percent) were migrants, 6 (7 percent) Injection drug users and 2 (2.4 percent) male homosexuals. About 21(22 percent) have acquired the virus during their stay in other cities of Pakistan, Sexual intercourse appears to be the main risk factor of transmission of virus along with the contaminated syringes and contaminated barbers' shaving tools. HIV has been on a rise in AJK due to the concealing behavior of patients contracting from overseas in particular from Middle East. It is crying need of the hour to create awareness about HIV along with its different risk factors among the risk groups of society, in addition HIV screening of all the migrants at the time of their arrival in the country must be made mandatory to inhibit the spread of this deadly virus.

**IN VIVO ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST
PSEUDOMONAS AERUGINOSA IN LABEO ROHITA**

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The emergence of diseases in aquaculture and development of antibiotic resistance is a global issue that threatens economic status of aquaculture sector. The excessive use of antibiotics contributes to this problem as the genes of antibiotic resistance can be transferred between the bacteria in fish. Metallic nanoparticles can serve as future substitutes for some conventional antibiotics because of their antimicrobial activity. The aim of this study was to evaluate the antibacterial action of silver nanoparticles against *Pseudomonas aeruginosa* bacteria in *Labeo rohita*. A 28-days trial was carried out. Fishes were allocated in four different experimental groups: Control group, INF (*P. aeruginosa* infected group), AgNP's (25µg/ml of Silver nanoparticles exposed) and AgNP's+INF (Combined treatment 25µg/ml of AgNP's and *P. aeruginosa*). After the completion of trial period, Change in RBC's, WBC's, Total serum protein and histology of kidney were studied. Result of this study revealed a non-significant ($P>0.05$) change in RBC's, TLC and in serum Total protein concentration in AgNP's exposed fish. Even no sever damage was observed in histomorphology of kidney. In bacterial intoxicated fish above mentioned parameters showed drastic changes in RBC's, TLC and Total serum Protein level, as compared to control group. Histo-morphology of kidney was also affected by bacterial infection. However, bacterial intoxicated fish treatment with silver nanoparticles showed marked recovery trend in all above mentioned parameters. Histo-morphology of fish kidney also showed marked recovery signs. The obtained results suggested that silver nanoparticles have an antibacterial activity and potential to boost up fish immunity against bacterial pathogen.

**PREVALENCE AND RISK FACTOR OF RHIPICEPHALUS BOOPHILUS IN BUFFALOES
FROM TANDO ALLAHYER, SINDH, PAKISTAN**

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Considering the economic impact of various tick's specie on livestock. The present study was projected the infestation rate of *Rhipicephalus boophilus* on Buffaloes from different localities of Tando Allayer April to

September 2017. Total 653 Buffaloes was randomly examined from different houses of buffaloes, Kacha and cemented houses with the help of forceps. The overall *Rhipicephalus boophilus* infestation rate is higher in kacha houses 81%. The highest and lowest prevalence was reported in the month of September (69.9%) and lowest in the month of April (37%). Based on seasonal prevalence. The highest *Rhipicephalus boophilus* infestation was found in rainy and humid season (61.4%) followed by summer (50.9%) while lowest found in April hot and dry condition. The highest age wise prevalence was notice in the young ones 75.17%, followed by growing 61.93% and lower in adult 27.7%.

DISEASE BURDEN OF MALARIA IN DISTRICT SANGHAR, SINDH, PAKISTAN

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Malaria is one of the worldwide health problems and imposes a major burden on health in under developed countries of the world. Half of the world population is at risk of malaria with an estimated 250 million clinical cases and nearly one million deaths were reported in 2006. Pakistan is among 109 countries with endemic malaria. Malaria transmitted through vector *Anopheles* mosquito which transfers *Plasmodium vivax* and *Plasmodium falciparum*. *Anopheles* mosquitoes belong to order Diptera and family culicidae, sub family anophelinae. Present study is carried out during April-November 2017 in three talukas of district Sanghar. Data of malaria were collected from taluka hospitals of different localities of district Sanghar. A total of 16771 suspected cases of malaria were examined. Out of these 321 were found positive. Among these positive cases, 153 male and 168 female were observed. Out of these 313 were identified as *Plasmodium vivax* infection while only 08 cases with *Plasmodium falciparum*. These cases indicate that *Plasmodium vivax* were dominant over *Plasmodium falciparum*.

INCIDENCE AND RISK FACTORS OF HEPATITIS B AND C AMONG UNIVERSITY STUDENTS POPULATION IN NEELUM VALLEY OF AZAD JAMMU AND KASHMIR

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Hepatitis is a medical condition generally known as the enlargement of liver. Hepatitis B and C are deadly blood born infection of liver. More than 200 billion people of the world have been infected with hepatitis B and 270-350 million with HCV. In the present study 500 young students were screened out of which 200 were the orphan students and 300 were the university students.. The positive subjects for virus-related antigen and antibody were further confirmed for viral RNA (for HCV) and DNA (for HBV) in the blood by using polymerase chain reaction (PCR) amplification. HBV infection was more prevalent (75 percent) as compare to HCV (25 percent). Among HBV positive subject 83 percent were male and 17 percent female. In present study, the prevalence of hepatitis is 87 percent in male and 13 percent in female. While among HCV positive subject 100 percent were male and. The age group 18-25 was found predominately for both HCV and HBV. Genotype 3a was more prevalent among HCV positive subjects. Dangerously high Infection rate of Hepatitis B and C in the Students indicates an alarming health situation. So the authorities should pay attention on ever increasing hepatitis. People should be given a proper awareness, timely diagnosis, treatment and prevention against these deadly viral diseases.

INCIDENCE AND RISK FACTORS OF HEPATITIS B AND C AMONG UNIVERSITY STUDENTS POPULATION IN MUZAFFARABAD DIVISION OF AZAD JAMMU AND KASHMIR

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Hepatitis is a medical condition defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. Hepatitis B and C are the most common blood-borne liver infections worldwide. According to recent estimates, 270-300 million people worldwide are infected by hepatitis C virus (HCV) and more than 2 billion have been infected with the hepatitis B virus (HBV). Transmission of these viruses is carried out by exposure to infectious blood or fluids containing blood. In the present study, 4781 students from Muzaffarabad, Neelum and Hattian Bala were screened for hepatitis B and C prevalence using Immuno-Chromatography Kits & ELISA. The positive subjects for virus-related antigen and antibody were further confirmed for viral RNA (for HCV) and DNA (for HBV) in the blood by using polymerase chain reaction (PCR) amplification. The HBV infection was found to be the predominant liver infection in the population which was 1.5% of the positive cases, as against 0.8% of HCV. Among the HBV-positive subjects, 84.6% were male and 15.4% were female. Similarly among the HCV-positive subjects, 100% were male and no one was female. The age group 16-30 was found predominantly for both HBV and HCV. No co-incidence of HBV and HCV was found in any subject. The genotype 3a and an untypable genotype of HCV has equal ratio of 50%. Risk factors were analyzed through fulfillment of risk factor assessment questionnaire. Use of injections, sharing of personal belongings and dental surgery were found predominant among all risk factors. Awareness of more than 0.5 million individuals has been done through their direct and indirect involvement in awareness campaign. Dangerously high Infection rate of Hepatitis B and C in the Students indicates an alarming health situation. So the authorities should pay attention on ever increasing hepatitis. People should be given a proper awareness, timely diagnosis, treatment and prevention against these deadly viral diseases.

INCIDENCE AND RISK FACTORS OF HEPATITIS B AND C AMONG UNIVERSITY STUDENTS POPULATION IN POONCH DIVISION OF AZAD JAMMU AND KASHMIR

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Hepatitis is a viral infection which disrupts liver functions and structure. There are five types of hepatitis viruses with a great morphological and functional difference. These are symbolized as hepatitis A, B, C, D, and E. Out of these viruses HBV and HCV are more dangerous and causing severe illness. Globally about 1.2 million people die annually due to HBV infection while HCV affects 170 million people of the world population. Hepatitis B and C viruses transmit from one infected person to another by contaminated blood and other body fluids. In current study we targeted 899 students and employees of AJK University belonging to Poonch division of AJK. Firstly, their blood samples were screened by simple ICT method. The samples which were diagnosed positive further treated with ELISA. Out of 47 ICT confirmed only 29 samples were positive after ELISA. Further confirmation was done by PCR technique. Total 23 individuals were positive for HBV and HCV after PCR. According to PCR estimation total hepatitis prevalence was 2.5% in whole division while HBV prevalence was 43.4% and HCV prevalence was 56.5%. Targeted population was belonging to different age groups like 15-20, 21-26, 27-32 and above 32. Significant prevalence was noticed in the students of age

group 21-26. Some risk factors are also effective for the prevalence of HBV and HCV some of them are dental surgery, some major or minor surgeries and blood transfusion.

THE PREVALENCE OF HYDROPERICARDIUM IN THE COMMERCIAL BROILER FARMS OF HYDERABAD AND ITS ADJOINING AREAS

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The Hydropericardium(HPS) syndrom disease commonly called Litchi Heart disease, primarily of broiler birds and mortality rate as higher as 75%, 3 to 6 week old age group birds are affected due to disease and causing a sever risk to poultry producers mainly in the broiler industry in Pakistan. Clinical diagnosis of the disease before the occurrence of mortality is difficult, since the birds do not show any specific clinical signs. The present study was carried out to investigate the occurrence of Hydropericardium disease in commercial broiler farms of Hyderabad and its adjoining areas i.e Hyderabad, Hala, Tando Muhammad Khan, Tando Allah Yar, Matyari. Data was randomly collected from study areas and Official record of the project director poultrydisease diagnostic labortary Hyderabad Sindh. According to the results , the birds affected due to HPS among all study areas remarkably higher (25.16 ± 3.46) in forms of Hyderabad and lowest from Matyari (12.33 ± 1.62) while highest mortality observed at the farms of Hyderabad (22.08 ± 2.82 with 87.74 %) lowest recorded broiler farms of Matyari (10.33 ± 1.25 with 83.78%), it was further noted that among the affected birds the rate of suvival broiler birds was observed highest at Hyderabad (3.08 ± 0.81 with 12.25%) and lowest from Matyari (2.0 ± 0.56 with 16.21 %). Present result shows highest number of birds affected, died and survived at hyderabad and lowest at matyari as compared to other study areas.

PREVALENCE OF HEMOGLOBINOPATHY IN PATIENTS OF ISLAMABAD CAPITAL TERRITORY, PAKISTAN

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Hemoglobinopathy is a common genetic disorder which results in abnormal structure of the hemoglobin molecule affecting its physiology. Objective of current research was to study the prevalence of hemoglobinopathies in population of Islamabad Capital Territory. A total of 118 blood samples were analyzed using Hemoglobin electrophoresis kit. From each patient, 2-3 ml of blood was collected in an EDTA tube and was stored for one week at 2-3 degree centigrade. The complete blood profile and peripheral film of sample was then taken. Hemolysates of samples were prepared on the same day the electrophoresis was run. The samples were loaded in wells of electrophoresis machine. After electrophoresis, the graphs were obtained on the paper. From reference values, the thalassemia and anemia disease patients were figured out. Of 118 total samples, 60 show haemoglobin disorder with over all prevalence rate of 50.84. While 24 cases showed beta thalassemia at the rate of 20.33 %. Beta thalassemia was found to be more common in males 41.87 % as compared to the female population (17.63%) while 36 patients were found (female 43.40% ; male 15.22%). Haemoglobin electrophoresis and complete blood profiles were used for the diagnosis of Haemoglobinopathies. Cousengenious marriages are common reason to increase its prevalence. Through awareness and premarital and prenatal screening its prevalence can be reduced.

EPIDEMIOLOGICAL STUDIES OF HBV AND HCV IN HIGH RISK POPULATION OF JAIL PRISONERS AND INTRAVENOUS DRUG USERS

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The objectives of the study were to prevalence of HBV and HCV infection among the high risk groups of transgender population, intravenous drug users and jail prisoners of the two Districts of Azad Jammu and Kashmir and to find out the predisposed risk factors that contribute to the alleviated level of the viral infections among these specific study groups. A total of 210 prisoners were included in the study, of which 121 individuals were from Central Jail Mir pur including 112 males and 9 females. There were 89 individuals from prison of District Kotli including 80 males and 9 females. Blood samples (5 ml) were taken from prisoner inmates and Chemiluminescent micro particle immunoassay (CMIA) was performed for clinical diagnosis of HBsAg anti-HCV antibodies using Abbot Architect plus i2000sr in Pakistan Institute of Medical Sciences Islamabad (PIMS). Among the jail prisoners, 17 (8.09%) and 11 (5.23%) cases were found positive for hepatitis C and hepatitis B infection respectively. Among those infected prisoners, 85.7% were intravenous drug users showing strong positive correlation ($r=0.994$) between intravenous drug users and hepatitis infection. It is concluded from the present study that prevalence of hepatitis B and C infection is higher among high risk groups i.e. jail prisoners and intravenous drug users.

PATHOGENICITY AND IMMUNOSUPPRESSIVE EFFECT OF DIFFERENT VACCINAL STRAINS OF INFECTION BURSAL DISEASE VIRUS IN BROILER CHICKEN

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The current experiment was conducted to compare the efficacy of different of Infectious Bursal Disease (IBD) vaccines having different IBD virus strains against field virus. A total of two hundred, one-day old broiler chickens were divided into four groups (50 birds each group). Group A was subcutaneously vaccinated with immune complex vaccine at 1 day of age. The groups B and C were vaccinated with live IBD vaccines D78 and 228E at 20th and 16th day of age according to Deventer formula. The blood samples were taken randomly from five birds of each group at day 7, 14, 21, 28 and 35 to evaluate anti-IBDV antibody titer by ELISA. Five birds from each group were killed on days 14, 21, 28 and 35 to check bursa to body weight ratio, bursometry, gross and histopathological lesions scoring. Ten birds from each treatment group were challenged with virulent field IBDV on 28 day. On day 35 significantly ($P<0.05$) higher ELISA antibody titer was observed in group A vaccinated with immune complex vaccine. Bursa to body weight ratio of group A was significantly higher ($P<0.05$) as compared to other vaccinated groups. Mild to moderate histopathological lesions such as lymphocytic depletion, epithelial necrosis and mononuclear cells infiltration, fibrous tissue proliferation and edematous fluid were observed in vaccinated groups. The live and immune complex IBD vaccine induced adequate protection after challenged. The high mortality and morbidity observed in control group D1 in one week of challenge. The morbidity rate in group A1 and Group C1 was 90% and in group B1 was 70%. It concluded that conventional live IBD vaccines cause more severe damage in bursal follicles as compared to immune complex vaccine. Immune complex vaccine can be safer for day-old chick regardless of maternal derived antibody titers.

**CLINICAL SIGNS AND SYMPTOMS ASSOCIATED WITH HUMAN BRUCELLOSIS
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Brucellosis is a worldwide zoonotic infection that has different modes of transferring from animals to humans such as consumption of unpasteurized dairy products and contamination with discharged fluids during parturition. By improving diagnostic and treatment procedures brucellosis can be controlled and economic losses can be reduced. The present study was conducted to evaluate brucellosis related signs and symptoms in seropositive human being in district Jhelum, Pakistan. A total three hundred blood samples were collected from different villages of district Jhelum. Serum samples were screened using rose Bengal plate test and seropositive samples were further correlated with brucellosis related signs and symptoms. Eight samples were positive for brucellosis antibodies on the basis of RBPT. Joint pain and sweating most prominent signs and symptoms in all seropositive brucellosis cases. Moreover, fever, headache, back pain, weight loss fatigue and chills were also reported from multiple seropositive patients. Brucellosis is a very hazardous disease but it is considered as an ignored disease in Pakistan. There should be proper treatment for brucellosis in Pakistan as well as public awareness should be provided about brucellosis especially in villages because in rural areas most of the people are in direct contact with animals.

**COMPARATIVE ANALYSIS OF LOCALLY DEVELOPED VACCINES AND
MONENSIN AGAINST COCCIDIOSIS IN CHICKS****Naveeda Akhtar Qureshi*, Wajiha and Amina Malik***Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad. Pakistan*

Avian coccidiosis the most detrimental and lethal disease of poultry is caused by obligatory intestinal apicomplexan protozoan parasite of genus *Eimeria*. Expanded use of anticoccidial drugs for controlling coccidiosis has resulted in the development of drug resistant strains of coccidian parasites. Due to drug resistance interest has been taken in egg adapted vaccines for efficient and inexpensive control of coccidiosis. In the present study three types of eggs adapted vaccines; egg adapted gametocytes, sonicated gametocytes and formalin inactivated gametocytes has been developed for controlling coccidiosis. Comparative efficacy of monensin and egg adapted vaccines against coccidiosis was investigated. Total of ninety, day old chicks were divided into six groups (A-F). On 5th day of age, three groups were orally given developed vaccines at dose of 0.2mL. On 21st day of age all groups except negative control were orally infected with dose of 10,000 oocyst/chick. On 5th day of post-infection positive control group was given monensin at dose of 60mg/kg for comparative analysis with vaccines. Parameters such as histopathology, biochemical analysis, body weight gain, feed consumption, lesion score, bloody diarrhea and excreted oocysts were investigated. Blood samples were collected from each group on 5th and 15th day post-vaccination as well as on 7th day post-infection for Indirect hem agglutination test. Gametocytes vaccinated and monensin treated group had significantly higher body weight gain, feed consumption when compared with other vaccinated groups. Mild bloody diarrhea with lowest oocyst count was shown by gametocyte vaccinated and monensin treated groups with 100% survival rate. A significant increase in the level of uric acid, ALT, AST, ALP, creatinine and decrease in level of albumin was shown by formalin, sonicated gametocytes vaccinated group and non-medicated infected group. Indirect hem agglutination test for antibodies detection showed maximum concentration in gametocytes vaccinated compared to control group. Current investigation revealed that effect of live *Eimeria spp* gametocytes vaccine (experimentally developed) against coccidiosis was significantly more effective than formalin inactivated and sonicated gametocytes vaccines and monensin treated group.

ANTICOCCIDIAL SCREENING OF *SYZYGIUM CUMINI* AND *FICUS RACEMOSA* LEAVES EXTRACT FOR THE CONTROL OF COCCIDIOSIS IN BROILERS

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Avian coccidiosis one of the most common and economically devastating intestinal disease is caused by the protozoan parasite of genus *Eimeria*. For over 60 years, anticoccidial drugs have been used to combat infection but their extensive use has resulted in the development of drug resistant *Eimeria* strains. Because of drug resistance and costly vaccine interest has been taken in the use of plant secondary metabolites having antioxidant properties for efficient and inexpensive control of coccidiosis. The aim of present study was to figure out the effect of 70% ethanolic leaf extracts of *Syzygium cumini* and *Ficus racemosa* as coccidiostat in broilers compared with commercial drug (coccinil plus). A total of 162, day-old broilers divided into nine groups (A-I) with triplicate. Each bird (group A-H) was infected orally with 10,000 sporulated oocysts of *Eimeria* spp at 14th day of age. At 5th day post-infection birds of group A-G were orally supplemented with 10, 15, 20% extracts of *Syzygium cumini* and *Ficus racemosa* while group H being positive control was treated with 0.6gm/litre coccinil plus for a week. Throughout the experimental period the body weight gain, feed consumption, feed conversion ratio was investigated while bloody diarrhea and lesion score were investigated at the first and the second week post-infection. Oocysts excretions were recorded from 6th-10th day post-infection. Blood was collected from all groups for biochemical analysis including urea, uric acid, creatinine, albumin, ALP, ALT and AST. Groups treated with 15, 20% *S. cumini*, *C. fistula* and 0.6gm/litre coccinil plus had significantly higher ($P \leq 0.001$) body weight gain compared to infected non-medicated control group. Birds treated with 15% *S. cumini* and 20% *C. fistula* extracts showed lower oocysts per gram of faeces, mild bloody diarrhea with 100% survival rate compared to infected non-medicated group. Birds treated with 15, 20% *S. cumini*, *C. fistula* and coccinil plus showed decrease level of ALT, AST, uric acid and increase ALP, albumin, urea, creatinine level when compared with infected non-medicated and other concentrations treated groups. *Syzygium cumini* and *Ficus racemosa* leaves extract at dose of 15% and 20% w/v was effective in control of coccidiosis in broilers thus showing similar results as coccinil plus treated group.

COMPARATIVE EFFECT OF *GEOTRICHUM CANDIDUM* (QAUGC01) AND *BACILLUS CEREUS* ON GROWTH, IMMUNITY AND SURVIVAL OF *LABEO ROHITA* (HAM.)

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In order to evaluate the effect of locally isolated fungal and bacterial probiotic *Geotrichum candidum* (QAUGC01) and *Bacillus cereus* respectively on *Labeo rohita*, advanced fry with average body weight 2.35 ± 0.12 g were evenly distributed in four groups, each with three replicate. The control group (C) was fed 35% protein diet without any supplement while the three treatment groups (T₁, T₂, T₃) were fed basal diets supplemented with *G. candidum* and *B. cereus* alone and in consortia of both probiotics, each containing 10^9 CFU kg⁻¹ diet for a period of 11 weeks. Results indicated that both probiotics showed significant effect on growth performance, survival, and immuno-hematological indices. However, *G. candidum* alone most significantly ($p < 0.05$) improved the growth performance in terms of % weight gain, and specific growth rate, blood parameters such as RBC, WBC count, hemoglobin content, total serum protein and respiratory burst activity and protection against *Aeromonas hydrophila* infection followed by consortium of both probiotic as compared to *B. cereus* alone. Based on the results, it appears that the fungal probiotic *G. candidum* has more pronounced effects on growth, immunity and survival of *L. rohita*. Thus, suggest its application in aquaculture as feed additive for improving the production of this cyprinid species.

STUDY ON RISKS ASSOCIATED WITH GESTATIONAL DIABETES MELLITUS IN WOMEN VISITING DIFFERENT HOSPITALS OF LAHORE

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The study was performed to determine the risk factors of gestational diabetes in women. The study was conducted on 250 subjects between the age of 21-35 years which included 50 non-pregnant, 50 normal and pregnant and 150 gestational diabetics. Demographic measurements such as age, height, weight, blood pressure, family history of diabetes and eating habits. Blood samples were collected and transferred to laboratory to analyze the glucose levels. Analysis of variance (ANOVA) was applied on the parameters for the comparison of mean between groups. The socioeconomic status of subjects was low. With increasing age the risk of diabetes also increases. The gestational diabetics have family history of diabetes. The mean age in gestational diabetics was 28.15 ± 0.38 years while in non-pregnant and pregnant was 24.74 ± 0.53 years, mean BMI in gestational diabetics was 29.8 ± 0.48 kg/m² while in non-pregnant and pregnant was 25.43 ± 0.72 kg/m², mean of systolic blood pressure in gestational diabetics was 135.53 ± 1.02 mmHg while in non-pregnant and pregnant was 119.40 ± 0.72 mmHg. The diastolic blood pressure in gestational diabetics was 93.13 ± 0.82 while in non-pregnant and pregnant was 80.00 ± 0.00 mmHg and 75 ± 0.71 mmHg respectively. The mean glucose level in gestational diabetics was 224.8 ± 6.95 mg/dl while in non-pregnant and pregnant was 73.24 ± 2.26 mg/dl and 83.00 ± 1.45 mg/dl respectively. The mean HbA1c value in gestational diabetics was 11.77 ± 4.63 mmol while in non-pregnant and normal and pregnant was 4.19 ± 0.08 mmol and 4.56 ± 0.05 mmol respectively. The age, BMI, blood pressure, family history, glucose level and HbA1c value are high in gestational diabetics, as they were at a higher risk of developing gestational diabetes.

SEROLOGICAL INVESTIGATION OF *TOXOPLASMA GONDII* IN PREGNANT WOMEN OF DISTRICT DIR (LOWER), KHYBER PAKHTUNKHWA, PAKISTAN

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Toxoplasmosis is a transmissible protozoan disease among animals and human beings the causative agent for the disease is a protozoan parasite *Toxoplasma gondii* which inhibit inside the cells. Different serological tests like Lateral Flow Chromatographic Immunoassay (LFCI), Latex Agglutination Test (LAT) and Enzyme-Linked Immunosorbent Assay (ELISA) were used to identify particular antibodies (IgM and IgG) in the blood of infected patient. The aim of this study was to evaluate seroprevalence of toxoplasmosis among pregnant women, from seven Tehsils of District Dir (Lower) and compare different serological tests like Lateral Flow Chromatographic Immunoassay (LFCI), Latex Agglutination Test (LAT) and Enzyme-Linked Immunosorbent Assay (ELISA). A total of 350 blood samples from seven Tehsils (50 from each Tehsil) were collected. The overall seroprevalence of toxoplasmosis was noted 17.14% (n=60/350) by LFCI, 19.42% (n=68/350) by LAT and 23.42% (n=82/350) by ELISA among the pregnant women. Variations resulted among seven (7) Tehsils of District Dir Lower; the highest prevalence of toxoplasmosis was noted in Tehsil Munda (36%) followed by Tehsil Lal Qilla (30%), Tehsil Samarbagh (26%), Tehsil Balambat (22%), Tehsil Khall (20%), Tehsil Timergara (18%) and lowest in Tehsil Adenzai (12%). From the present study it is concluded that the highest accuracy was shown by (ELISA) and lowest accuracy was shown by (LFCI) so instead of LFCI and LAT used ELISA for accurate investigation of toxoplasmosis among pregnant women.

DETECTION OF *LAS R* AND *LAS I* GENE IN *PSEUDOMONAS AERUGINOSA* ASSOCIATED WITH PULMONARY INFECTIONS

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P.aeruginosa is the most common prevalent pulmonary pathogen of human causing successfully opportunistic infections in immune suppressed patients of every age group and evident as a first or second major pathogen in most researches. It is the most lethal persistent pathogen of acute ventilator acquired pneumonia (VAP) and chronic Cystic fibrosis (CF) disease. Globally every year it results in major morbidity and mortality because of high recurrent significant infections rate and extensive virulence factors. Quorum sensing system of *P.aeruginosa* is a global regulator of almost all genes of virulence factors which directly relates to its pathogenicity. Both gene *lasR* and *lasI* of *P.aeruginosa* are component of *las* system of quorum sensing which regulates almost all genes of virulence factors. The recent study was designed to detect pulmonary infections associated genes *lasR* and *lasI* in *P.aeruginosa* that is responsible for pulmonary infections. The sampling was done during January to May 2015, during this period total 105 samples out of which 45 early morning ETT secretions (Endotracheal tract) and 45 Blood samples from patients of acute Ventilator associated pneumonia (VAP) from CICU/SICU and 15 bronchoscopy samples of chronic Cystic fibrosis (CF) patients of Paediatric ward were collected from Mayo hospital Lahore. Total 9 strains of *P.aeruginosa* was selected as most virulent strains after confirmatory test of antibiotic sensitivity and virulence assays. Later these pathogenic strains of *P.aeruginosa* were used for ribotyping of genes *lasR* and *lasI*. PCR products results and sequence analysis results after BLAST at NCBI confirmed 100% presence of both genes *lasR* and *lasI* in the genome of *P.aeruginosa* strains which are involved in multiple virulence factor and responsible for pulmonary infections. In the recent study the contribution of *lasR* and *lasI* gene of pathogenic *P.aeruginosa* responsible for pulmonary infections was investigated.

PREVALENCE OF PNEUMONIA IN CHILDREN UNDER TEN YEARS OF AGE IN DISTRICT KARAK, KHYBER PAKHTUNKHWA, PAKISTAN

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Pneumonia is a common illness affecting approximately 450 million people every year and occurring in all parts of the world. It is a major cause of death among all age groups, resulting in 1.4 million deaths in 2016 (7% of the world's yearly total). Rates are greatest in children less than five years and adults older than 75 years of age. In the present study 400 blood sample were collected from children of district Karak and were tested for pneumonia out of which 120 (27.50%) were found positive. Male were found to show high positivity as compared female children. The disease was found to be more in children residing in rural area (61.67%) compared to the children of the urban (38.33%) area. There is an urgent need for local action to address the disease and start to create awareness among public and provide proper medication and health facility regarding this disease.

EROPREVALENCE AND ASSOCIATED RISK FACTORS OF HEPATITIS E VIRUS AMONG PREGNANT WOMEN

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The primary hepatotropic viruses (hepatitis A, B, C, D and E virus) are the most common cause of acute liver disease. In developing countries the majority of these cases are caused by the hepatitis E virus (HEV).

Hepatitis E is enterically transmitted, causing a self-limiting disease similar to hepatitis A. It is endemic in most developing countries, where it causes major epidemics and sporadic cases. In the present study, 200 blood samples were collected from pregnant women randomly in different health centers of both urban and rural area of district Lakki Marwat, southern Khyber Pakhtunkhwa, in a span of six months from February 2016 to July 2017. Sera samples were screened for rubella IgG and IgM antibodies by ELISA (enzyme linked immunosorbent assay) technique. Our finding revealed that only 5/200 (2.5%) were positive for IgM and 11/200 (5.5%) for IgG. Higher age women were more positive than lower age. Low income status women were more positive for both IgG (6.78%) and IgM (3.38%). Women of the rural area and illiterate were also more susceptible for hepatitis E virus. A lot of women were antibodies positive against HEV infection suggestive of putting many women at high risk to HEV infection. Key step should be taken by the Government to address this disease in pregnant women. Also there is need to create awareness among pregnant women to tackle this disease.

COMPARATIVE STUDY OF VARIOUS SCREENING TESTS TUBERCULOSIS AND ITS EFFICACY IN BOVINE ANIMALS

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The aim of this study was to identify bovine tuberculosis at farm in poor setting resources to compare efficacy of various screening test by fecal, oral and tuberculin in animal herd at live stock farm Islamia university of Bahawalpur. The Prevalence of the bovine tuberculosis was 20.0 % by fecal method and 10.0 % by oral method was 6.7 % through tuberculin test. The prevalence in the farm workers were also negative, Hence Bovis between farmers were not found and their cattle were found high risk of BTB in non-descriptive cattle

DETECTION AND CHARACTERIZATION OF *WOLBACHIA* IN VARIOUS INSECTS

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Wolbachia based diseased vector control approaches have been purposed as a mean to augment the prevailing Dengue vector control strategies. Therefore, it is essential to have thorough knowledge about the prevalence and *Wolbachia*-host (vector) interaction. This study was performed to detect and characterize the local *Wolbachia* strain in 2 mosquito species (*Culex quinquefasciatus*, *Aedes albopictus*) and one species of fruit flies (*Drosophila simulans*) collected from different indoor and outdoor sites of Lahore, Pakistan. For this purpose, twenty insect pools from each sample containing 20 pairs of ovaries were examined for the presence of *Wolbachia*. gDNA was isolated using standard procedure. DNA was amplified by PCR using gene specific primers (*wsp* and *ftsZ*). Ten pools of *Cx. quinquefasciatus* (50%) were found to be positive for *Wolbachia*. NCBI Blast of *Wolbachia wsp* sequence isolated from *Cx. quinquefasciatus* showed 100% homology to the *wPip* isolate reported in U.K. belonging to super group B. *Wolbachia ftsZ* sequence showed 100% homology to the *wPip* isolates from *Cx. quinquefasciatus* and other related species belonging to *Wolbachia* super group B reported in USA, U.K and Argentina. All pools of *D. simulans* (100%) were found to be positive for *Wolbachia*. NCBI Blast showed 99% homology to the *wRi* isolate from different *Drosophila* species reported in USA, China, Austria and Sweden. *FtsZ* isolate of *wRi* also showed 99% homology to the *Wolbachia* isolate of *D. simulans* reported from Sweden. Phylogenetic analysis showed that it was closely related to *wRi* and the super group A. In *Ae. albopictus*, all the twenty pools (100%) were found to be positive with *wAlbA* and *wAlbB*. NCBI blast of the *wsp-A* and *wsp-B* isolates showed 99% and 100% homology to the *Wolbachia* isolate of *Ae. albopictus* from India and USA respectively. *FtsZ* isolate of *wAlbB* showed 100% homology to the *wAlbB* from USA. Phylogenetic analysis showed that A and B *Wolbachia* isolates in current study were clustered in A and B super groups already reported from different countries.

IMMUNOSORBENT SURVEY OF RUBELLA VIRUS INFECTION AMONG CHILDREN OF SOUTHERN KHYBER PAKHTUNKHWA, PAKISTAN**Aamir Khan, Syed Ishtiaq Anjum, Hikmat Ullah Khan and Kalim Ullah****Department of Zoology, Kohat University of Science and Technology, Kohat-26000, Khyber Pakhtunkhwa, Pakistan*

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Rubella (initially known as German measles) is associated with 80% risk of usually multiple congenital abnormalities if acquired in the first 12 weeks of pregnancy. A total of 200 blood samples were collected from small children randomly in different health care-centers/hospitals of both urban and rural area of district Lakki Marwat, Khyber Pakhtunkhwa. Sera samples were screened for rubella IgG and IgM antibodies by ELISA (enzyme linked immuno-sorbant assay) technique. Our findings revealed that 28% pregnant women were positive for IgG and 11% for IgM. Higher sero-positivity rates were found in the children of age group 4-6 years. Our result also indicated that children having low income status showed highest positivity rate for both IgG and IgM antibodies. Children residing in rural area showed higher sero-positivity rate (IgG=16% and IgM=7.5%) than urban area (IgG=12% and IgM=3.5%). A lot of women were found antibodies positive against RUBV infection suggesting of putting many unborn babies at high risk to rubella infection. Key steps are necessary to be taken by the Government to address this disease in pregnant women.

VITAMIN D RECEPTOR GENE POLYMORPHISM: A STRONG PREDICTOR OF OSTEOARTHRITIS ONSET**Saira Kainat Suqaina¹, Nadeem Sheikh^{1,2,*}, Maryam Mukhtar¹, Sabeen Nazir¹, Mehwish Shehzadi¹, Rabia Mehmood¹ and Naz Fatima¹**¹*Department of Zoology, University of the Punjab, Lahore, Pakistan.*²*Center of applied molecular Biology, University of the Punjab, Lahore, Pakistan.*

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Osteoarthritis (OA) is a non-inflammatory disease characterized by mutual interaction of hormonal imbalance, environmental factors and genetic polymorphism. Several genes were reported as susceptible risk factors in OA onset including Vitamin D receptor gene (VDR). Therefore, the present study was conducted with an aim to identify polymorphism on exon 2 of VDR gene in OA patients of Lahore division. For this purpose, a case control study was conducted on 30 patients and 30 control subjects. DNA was extracted by the modified Organic method and desired DNA fragment was amplified by PCR. Genotypes were identified by direct sequencing technique. Genetic polymorphism was identified on rs2228570 polymorphic site and was significantly associated with OA in studied population at both allelic as well as genotypic level. The polymorphism on rs2228570 polymorphic site led to the change of Tryptophan into Arginine. In conclusion, VDR gene polymorphism was identified as susceptible region for OA onset in population of Lahore division.

4. MICROBIOLOGY

FIMBRIAE ADHESIONS OF ENTEROTOXIGENIC *ESCHERICHIA COLI* ARE ESSENTIAL FOR ATTACHMENT AND BIOFILM FORMATION

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Enterotoxigenic *Escherichia coli* (ETEC) strains are leading causes of childhood diarrhea in developing countries. Adhesion is the first step in the pathogenesis of ETEC *E. coli* infections and ETEC pili designated colonization factor antigens (CFAs) are believed to be important in the biofilm formation, colonization and host cell adhesions. As a first step, we have determined the biofilm capability of enterotoxigenic *E. coli* expressing various types of pili (CFA/I, CFA/II and CS2) and tip mutated piliated CFA/I strains. Further, enzyme-linked immunosorbent assay (ELISA) assay were developed to compare the binding specificity of CFA/I, CFA/II (CS1 - CS3) and CS2 of ETEC *E. coli*, using extracted fimbriae and fimbriated bacteria. CFA/II strain as well as extracted pili exhibited significantly higher binding both in biofilm and ELISA assays compared to non piliated and mutant/wild recombinant strains. This indicates that co-expression of two or more CSs in the same strain is more efficient in increasing adherence compared to those having one only. Significant decrease in binding specificity of CS2 strain with deleted cotD and *CfaE*-R181 tip mutant strain indicated the important contribution of minor tip proteins in adherence assays. In addition no effect was observed on agglutination of bovine erythrocytes in R181-CotD mutant strains of CS2 showed that minor tip protein may not be important as adhesions in these strains. Isolated CFA/I, CFA/II and CS2 pili as well as bacteria expressing particular antigens on their surface bound to several intestinal cell membrane structures and play a significant role in host cell colonization. In summary, our data suggest that pili, their minor subunits are important for biofilm formation and adherence mechanisms. Overall, the functional reactivity of strains co expressing various antigens, particularly minor subunit antigen observed in this study suggest that fewer antibodies may be required to elicit immunity to ETEC expressing a wider array of related pili.

IDENTIFICATION OF A NOVEL GOLD SPECIFIC HOMEOSTATIC SYSTEM IN *KLEBSIELLA PNEUMONIAE*

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Metals serve as micronutrients and are classified as essential (Na, K, Mg, Ca, Cu, Zn) and non-essential (Cd, Hg, Au, Ag, Al) depending upon their importance in biological systems. The increased level of both essential and non-essential metals can alter the cellular homeostasis. *Klebsiella pneumoniae*, an enterobacter, has Cue and Cus regulatory systems for combating the toxic effects caused by coinage metals (Copper, Silver and Gold). However, gold-specific regulatory system has not been reported yet. The present study aims to elucidate the gold-specific homeostatic mechanism in *K. pneumoniae*. *K. pneumoniae* cells were grown in LB broth for 2 hs and metals (Cu and Au) were added with final concentration of 4 mM and 0.1 mM, respectively. In control sample no metal was added. After 4 hs of metal addition, cells were pelleted down and protein profile of each sample was compared with control on SDS-PAGE.

EVALUATION OF ANTIBIOTICS RESIDUES IN BROILER CHICKEN COLLECTED FROM DIFFERENT CITIES OF PUNJAB, PAKISTAN

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The poultry meat is the second most eaten food all over the world and the production of poultry meat has increasing tremendously all over the world. To prevent the diseases and maintain the continuous supply to fulfil the demand of poultry meat for human consumption, antibiotics are used in Pakistan. In this research work, 36 samples of broiler meat (18 muscles and 18 liver samples) were collected from six different cities of Punjab, Pakistan. viz; Islamabad, Faisalabad, Kasur, Multan, Lahore and Bahawalnagar. The antibiotics were detected using HPLC-UV detector. Two antibiotics; levofloxacin and ciprofloxacin were detected from the chicken tissues. The presence of antibiotics in broiler muscles were observed in 33% samples belonging to Islamabad, Faisalabad, Kasur and Bahawalnagar, whereas, in the case of liver both antibiotics were detected from all localities. The antibiotics concentrations in the muscle samples from Lahore was below detection limits. Highest concentration in the muscle samples were detected in Bahawalpur (levofloxacin 41.95µg/g and ciprofloxacin 23.74µg/g). In case of liver samples, antibiotics were detected in all samples except Kasur. The mean concentration of antibiotics residues in muscles and liver samples were below the permissible limits (200µg/g for liver and 100µg/g for muscles) described by European Union-maximum residual limit (EU-MRL) except the liver sample from Bahawalnagar (ciprofloxacin; 249.86µg/g). The chicken muscles are the major part and preferably consumed by the local community. On the basis of the present results highlighted that broiler meat is safe for human consumption. This study was small scale; however, more research is required to confirm on large scale.

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *TRILLIUM GOVANIUM* EXTRACT, THEIR CHARACTERIZATION AND ASSESSMENT OF ANTIBACTERIAL, CELL VIABILITY, BIOFILM INHIBITION AND PHYTOCHEMICALS

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There is a severe concern about evolving contagious infections and the rising drug resistance in bacterial pathogens. Silver nanoparticles are considered as most effective nanoparticles due to the good antimicrobial efficacy. The current study was aimed to synthesize the silver nanoparticles using *Trillium govanianum* characterize and investigate their antibacterial activities against human-associated bacterial pathogens viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* through agar well diffusion method, MTT assay, and anti-biofilm assay. Characterization of green synthesized nanoparticles was done through UV-viz spectrophotometry, Scanning Electron Microscope, and Fourier-transform infrared spectroscopy. For antibacterial activity of extract and silver nanoparticles agar well diffusion method with different concentrations of plants was used. Biofilm formation was measured using the microtiter dish assay system, MTT assay was used for estimating the viability of bacterial Cells. Presence of Phytochemicals were also analysed. Silver nanoparticles showed maximum zone of inhibition against *E.coli*, *S. pyogenes* and *S. aureus* 8.33 ± 0.06 mm, 7.00 ± 0.00mm and 6.66 ± 0.06mm respectively as compared to this very low zone of inhibition showed by these pathogens against extract, 5.00 ± 0.00 mm, 0.00 ± 0.00 mm and 3.00 ± 0.00 mm. Remaining 4 pathogens showed medium zones against both extract and Silver nanoparticles. Cell viability assay, antibiofilm assay, supported the results of agar well method. Many natural biomolecules: saponins, tannins, alkaloids, free amino acids, quinones, phenols, terpenoids, steroid and glycosides were found in *T. Govanianum* extract. It is concluded that silver nanoparticles possessed antimicrobial activity against various tested pathogens. Silver nanoparticles could be used against various infectious agents to overcome the problem of multidrug resistant developments.

CONSERVED DOMAINS IN EUKARYOTIC-LIKE PHENOL HYDROXYLASE (PHE) OF *CORYNEBACTERIUM GLUTAMICUM*

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Phenol hydroxylases catalyze the initial reactions in phenol biodegradation and detoxification by converting it into catechol, which is then subjected to intra or extra diol ring cleavage by dioxygenases in aerobic microorganisms. Two-component phenol hydroxylases are widely distributed in Gram-positive bacteria. However, a single-component, eukaryotic phenol hydroxylase (Phe) has been identified in *Corynebacterium glutamicum*. Phe (NCgl2588) contains 627 amino acid residues. NCBI's conserved domain database (CDD) was accessed to define conserved domains and residues while Jpred v.4 server for secondary structure prediction. Phe contains a Rossmann-fold NAD(P)H/NAD(P)(+) binding (NADB) domain characteristic of numerous redox enzymes. H-bonding in a turn between first beta strand and the helix of Rossmann-fold facilitates NAD binding. The consensus binding pattern GXGXXG was located in this turn where G39 and G41 are involved in NAD(P)-binding and G44 facilitates close packing of the helix to the beta-strand. Typically, proteins in NADB family contain a second domain for specific binding of substrate for catalysis. This domain was also identified in Phe and it belongs to FAD-dependent phenol hydroxylase (PHOX) family that catalyzes the hydroxylation of phenol with the consumption of NADPH and oxygen. The functional unit of PHOX is a homodimer where seven residues are highly conserved and are involved in forming substrate and FAD/NAD(P) binding site. These are D477, T479, Y537, Y538, D541, T542 and V561. The C-terminal TRX-fold domain and these conserved residues are involved in forming the dimerization interface.

SEQUENCE ANALYSIS OF MARA FOR MULTIPLE ANTIBIOTIC RESISTANCE IN SEVEN GENERA OF THE FAMILY ENTEROBACTERIACEAE

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Bacteria can evade sudden appearance of antibiotics through stochastically entering a dormant, drug-tolerant state, which is caused by induced gene expression to generate a resistant phenotype. Cellular mechanisms such as DNA and protein synthesis are inhibited during dormancy and subsequently cells evade antibiotics that target cell growth. Previous studies have shown that MarA (the multiple antibiotic resistance activator) plays a significant role in multidrug resistance. MarA expression was heterogeneous within the population, which was correlated with transient antibiotic survival. The *marRAB* operon is autoactivated by MarA, autorepressed by MarR and indirectly repressed by the periplasmic protein MarB. In the present study, MarA proteins from seven representative genera (*Escherichia*, *Shigella*, *Salmonella*, *Citrobacter*, *Enterobacter*, *Klebsiella* and *Cronobacter*) of the family Enterobacteriaceae were selected for analysis. These are helix-turn-helix (HTH) proteins of AraC family transcriptional regulators, which control the expression of genes with diverse biological functions including metabolism, stress response and virulence. MarA proteins contain 124–129 amino acid residues (molecular weight 14.6–15.1 KDa) and 84% of them are conserved. Among 7 α -helices, helix 3, 5, 6 and 7 are fully conserved. The theoretical pI is in the range of 9.3–9.6. The sequences show high similarity (90.5–100%) with MarA of *E. coli* K-12 with fully resolved three-dimensional structure at 2.3 Å.

EVALUATION OF ANTIBACTERIAL POTENTIAL OF SELECTED PLANT EXTRACTS ON *E. COLI* PATHOGEN ISOLATED FROM URINARY TRACT INFECTION

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Urinary tract infections (UTI) are the second most common type of infection in humans. Different sex

and age group of people in many countries especially in developing countries is affecting by UTI each year. The ratio of infection is more among females higher (56.9%) than males (43.1%). The aim of present study is to evaluate the performance of antibacterial effect of plant extract against *E.coli* the most common agent for causation of urinary tract infection. *E.coli* show wide range of antimicrobial resistance against antibiotics. The use of plant extracts against infections is an old method but Plant extracts showed high sensitivity pattern for *E.coli* than antibiotics (i.e. ciprofloxacin, levofloxacin). Plant material (green tea, haldi, dar chini, ajwain, garlic) was collected from local market of Faisalabad. Plant material was passed through pretreated methods such as dried in oven at 45°C and milled. Natural material was treated with ethanol, distilled water and n-hexane. They were passed through rotary at 65,75, 100°C and saved at 4°C. A total 50 patients urine samples was collected from Allied hospital, Faisalabad then processed for isolation of *E.coli* on Eosine Methylene Blue agar. *E.coli* was identified by CLED, McConkey and nutrient agar method. Antibacterial activity of plants extracts was checked by disc diffusion method showing prominent zones of inhibition uptill 20 mm. Minimum inhibitory concentration (MIC) of extracts was also measured. Qualitative phytochemical analyses showed presence of alkaloids, carbohydrates, saponins, flavonoids, steroids/terpenes, tannins, resins, glycosides, and anthraquinones. Thin layer chromatography differentiated the chemicals present in extracts. The extracts of plants exhibit equally effective against *E. coli* of UTI which is suggestive to use against the urinary tract infection in efficient manner.

ANTIMICROBIAL EFFECT OF SELECTED PLANT EXTRACTS AGAINST FOOD POISONING BACTERIA

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Biological, chemical, and physical contamination of foods is a horrifying warning for the health and economic growth in developing societies. Dominance of many pathogens in several foods is common place in Pakistan. Food poisoning is considered as one of the most common cause of illness and death in developing countries. Prevention from food poisoning pathogens is usually gained by use of chemical preservatives which have negative impacts on human health, as well as due to the chemical application and chemical residues in food, the acquisition of microbial resistance of the used chemical takes place. As a result of this condition, there is a need to find potentially effective, healthier, safer and natural alternative preservatives. The aim of present study was the utilization of plant material as natural alternative preventives to control food poisoning diseases. Plant materials (cumin, onion, coriander seeds and fennel) were collected from local market in Faisalabad District Pakistan. First of all, Plant materials were dried through oven at 45°C and ground into a fine powder. The powder then treated with ethanol and distilled water. The plant materials were passed through the rotary to get plant extracts. Ethanolic extracts were prepared. Bacterial strains causing food poisoning were collected from the strain bank University of the Punjab Lahore. Plant extract were applied against bacterial strains causing food poisoning (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhi*) using agar disc diffusion technique showing prominent zones of inhibition ranges from 10 to 20 mm. Minimum inhibitory concentration (MIC) was estimated against these strains ranged from 2.5 to 5.0 mg/ml. Phytochemical screening of extracts detected the chemical constituents like flavonoids, alkaloids, glycosides, carbohydrates, phenols, fats, proteins. Thin layer chromatography of extracts differentiated the different chemicals. Thus the chosen plants proved to be effective against food poisoning bacteria and have potential to control the said disease.

UTILIZATION OF AGRO-INDUSTRIAL WASTE FOR BIOSTIMULATION OF BACTERIAL ISOLATES PRODUCING THERMOSTABLE CELLULASE ENZYME

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Pakistan is an agricultural country with tons of waste products as corn stovers, corncob, rice husk, wheat bran and cotton residues. Cellulose is the chief component of plant residues which is also a vital source for

industrial products as it is an energy rich compound but the remains of agriculture and industries have no better utilization and becomes heaps of wastes degrading environment. Agro-industrial wastes are potential candidate of cellulase production. Enzymatic hydrolysis is an economic process for conversion of cellulose to easily fermentable low cost sugars. The aim of present study is utilization of agronomic waste contents for yielding cellulase enzyme from the thermophilic bacterial isolates, which are eco-friendly and fulfill our increasing cellulase demands by utilizing low cost feedstock. Substrate samples (sugarcane bagasse, wheat bran, corn cob and rice husk) were collected from agro-industries and fields in Faisalabad District Pakistan. Agronomic waste materials were washed, dried in oven at 80°C and milled. The substrates were pretreated with hydrochloric acid for removal of lignin from lignocellulosic substrates. Pretreated wastes were analyzed by FTIR and XRD. Cellulolytic bacteria were isolated from the soil sample of rice field in new campus Government college University Faisalabad at elevated temperature (55°C). The isolates were further screened with iodine test for cellulase activity and enzyme was estimated by DNS method. Five isolates showed highest zone of clearance and enzyme activity, were identified by biochemical test and rRNA gene sequencing which showed mostly belong to genus *Bacillus*. To further increase the enzyme production optimization of thermostable cellulolytic bacterial isolates was performed through response surface methodology using different growth parameters. Pretreated wastes were utilized for production of thermostable cellulases in optimized conditions. Bioethanol production was estimated by utilizing wastes which showed their potential application in low cost bioenergy production.

COMPARATIVE GROWTH POTENTIAL OF THERMOPHILIC AMYLOLYTIC BACTERIA ON UNCONVENTIONAL FOOD WASTE

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Amylases play a vital role in industries such as food, fermentation, starch processing, textile and paper etc. Increasing amylases demand, high nutrient cost and environmental pollution have compelled utilization of agro-industrial residues as a low-cost feedstock for enzyme production. In present study α -amylase was synthesized from thermophilic bacterial isolate. For this study, soil samples were collected from agro-industrial waste dumping areas in District Faisalabad and processed for isolation of α -amylase producing bacteria at elevated temperature (55°C) and screening was done with starch hydrolysis test by iodine method. Twenty amylolytic strains were attained with prominent zone of clearance on starch. α -Amylase production was assayed by DNS method. Five amylolytic bacterial isolates giving prominent amylolytic production was identified by both biochemically and 16s rRNA gene sequencing technology that mostly belonged to *Bacillus* sp. Optimization was performed using Response Surface Methodology (RSM) by applying multiple process parameters such as inoculum age, inoculum size, nitrogen source concentration, temperature and pH. Unconventional food wastes (mango, potato, banana and lemon) were used for the low cost production of amylases. These wastes were collected from hostel of G.C. University Faisalabad. Different pretreated methods such as washing with distilled water, drying in oven at 80°C, grinding and acid hydrolysis was applied on these wastes before utilization. Pretreated waste was also analyzed by FTIR and XRD analysis. Potential thermostable amylolytic bacterial isolate showed promising results by utilizing wastes.

EVALUATION OF ANTIMICROBIAL AND PHYTOCHEMICAL POTENTIAL OF *PSIDIUM GUAVA* LEAVES THROUGH GC-MS AND HPLC

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Psidium guajava leaves are rich source of nutrients, antioxidants, phytoconstituents and biological active

compounds. The current study was proposed to investigate the amount of secondary metabolites like alkaloids, saponins, flavonoids, tannins and glycosides in organic leaf extracts of *P. gujava* through Gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) by qualitative as well as quantitative procedures. Moreover these secondary metabolites were also tested for their antimicrobial potential against two gram positive (*Bacillus subtilis* & *Staphylococcus aureus*) and two gram negative (*Escheria coli* & *Pasteurella multocida*) bacteria and three fungal species (*Asprrgillus niger*, *Fusarium solani* and *Aspergillus flavus*) which are known pathogens. Gas chromatography-mass spectrometry spectra of *P. gujava* essential oil contained major constituents such as Ca- Carophyllene 22.70%, α cubebene 11.2% and alpha-Humulene 5.91%. The ethyl acetate, methanol, n-hexane and chloroform extracts of *P. gujava* (Amrood) were tested for antibacterial and antifungal activities against all above mentioned microbes. Among all the tested solvent extracts, methanol and ethyl acetate extracts of *P. gujava* possessed maximum antimicrobial activity against tested bacterial and fungal strain's growth. These findings may provide raw data about the therapeutic potential of *P. gujava*.

ASSESSMENT OF BIOFILM INHIBITION AND CELL PROLIFERATION EFFECT OF *AMYNTHUS MINIMUS* AGAINST HUMAN-ASSOCIATED BACTERIAL PATHOGENS

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Development of microbial resistance to various existing antimicrobial drugs has become serious public health concern and the search for new classes of antimicrobial agents is task of the period. Therefore, scientists have begun to find new antibiotics from natural resources. Antimicrobial agents are limited from animal sources especially from earthworms. Current research focus on the extracts of earthworm specie *Amyntus minimus* to explore its antibacterial effect against seven clinical bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* through agar well diffusion method. Cell viability and antibiofilm effect were also done through MTT and crystal violet assays. It was observed that *Amyntus minimus* indicated the significant inhibition of all the tested pathogens. Synergistic effect with antibiotics also showed significant inhibition. The cell proliferation inhibition and biofilm inhibition effect of *Amyntus minimus* also supported the antibacterial effect. The findings of current study are more effective and would be useful for making potential antibacterial drug from earthworm against clinical bacterial agents.

ANTIBACTERIAL, BIOFILM INHIBITION AND CELL PROLIFERATION EFFECT OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING *AJUGA BRACTEOSA* AGAINST CLINICAL BACTERIAL PATHOGENS

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Synthetic antibiotics and the development of resistant strains are the major problems of the developing countries. Various strategies, treatments and preventions are available to combat the multidrug resistant bacteria. So, the current study was aimed to determine the antibacterial effect of biogenically synthesized silver nanoparticles using *Ajuga bracteosa*. Synthesis and characterization of silver nanoparticles of *Ajuga bracteosa* were done through biological, UV-viz spectrophotometry, Scanning Electron Microscope, and Fourier-transform infrared spectroscopy. The antibacterial activity was performed by agar well diffusion method.

Biofilm formation was measured using the crystal violet assay and the cell viability was determined by MTT assay. DPPH [(di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium] and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS+ free radical scavenging activity was carried out to evaluate the antioxidant potential of extracts and silver nanoparticles. The plant materials were assessed for photochemical screening. The indication of major phytochemical constituents was further confirmed by Thin Layer Chromatography. Direct bio-autography was also done *via* agar overlay technique. Nanoparticles showed significant effect against seven pathogens as compared to aqueous extract at the concentration of 0.1 g/ml. DPPH and ABTS scavenging activity showed the significant results in extracts as compared to nanoparticles. Study revealed presence of tannin, saponanins, alkaloids, phenols, terpenoids, steroids, quinones, glycosides, and amino acid phytochemicals. All nanoparticles showed more antibacterial effect compared antibiotics. TLC-bioautography, biofilm inhibition and cell proliferation inhibition effect supported the results of antibacterial activity. This study therefore, concludes that green synthesized silver nanoparticles have impact on treatment of various infectious diseases. An increasing awareness towards use of green route for synthesis of metal nanoparticles lead a desire to develop environment-friendly techniques.

ANTIBIOFILM AND CELL PROLIFERATION EFFECT OF *ALLOLOBOPHORA CHLOROTICA* EXTRACT AGAINST CLINICAL BACTERIAL PATHOGENS

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Earthworms have been used in medicine for various remedies. The aim of current research was to evaluate the antibacterial effect of earthworm species *Allolobophora chlorotica* extract against clinical bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The extract was prepared through maceration process. Methanol was used for the preparation of earthworm extract. Antibacterial activity was carried out using agar well diffusion method. On the other hand, synergistic effect of *Allolobophora chlorotica* and standard antibiotics was also done via agar disc diffusion method through agar well diffusion method. Cell viability and antibiofilm effect were also performed through MTT and crystal violet assays to confirm the antibacterial effect of *Allolobophora chlorotica* extract. The synergistic effect was observed when both antibiotic and extract was used. The range of recorded zone of inhibition was 11.66±0.0 mm to 33.0±0.0 mm. MTT and cell proliferation assay showed the reduction of bacterial growth. So, the results of current study were more effective and *Allolobophora chlorotica* extract may lead to the formulation of new antimicrobial drug.

MOLECULAR DETECTION OF NEW DELHI METALLO BETA-LACTAMASE (NDM) PRODUCING GRAM NEGATIVE BACTERIA FROM PATIENT SAMPLES IN LAHORE.

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The metallo-beta-lactamase (MBL) enzymes had the capacity to hydrolyze beta-lactam antibiotics. New Delhi metallo-beta-lactamase (NDM) is a novel type of MBL that inactivated all β-lactam antibiotics except aztreonam. The aim of this study was to detect the New Delhi Metallo beta-lactamase producing Gram-negative bacteria from patient samples in Lahore, Pakistan. About 180 bacterial strains were collected from various samples (pus, sputum, tracheal swab, urine etc) from PGMI during November 2016 to January 2017. Gram staining and biochemical characterization of these strains were done and identified as *E. coli*, *P. aeruginosa* and *K. pneumoniae*. Antibiotic susceptibility test was performed to determine sensitivity and resistance pattern to

different antibiotics. Results indicated highest resistance of to ceftriaxone with all strains and lowest resistance to cefepime, cefotaxime and imipenem with *E. coli*, *P. aeruginosa* and *K. pneumonia* respectively. For ESBL and MBL producing strains double disc synergy and EDTA tests were performed. It was noted that 72% of all bacterial strains showed ESBL production and 50% showed MBL production. The plasmid DNA was isolated by alkaline lysis method and NDM gene was detected by polymerase chain reaction. The desired *bla*_{NDM} gene band (621bp) were visualized on 1% agarose gel. The prevalence of NDM gene that causes resistance to antibiotic was 32%. It is concluded that resistance to antibiotics and isolation of bacteria producing NDM is increasing day by day. Therefore the rapid identification of strains expressing NDM is important to control their spread.

MICROBIOLOGICAL ANALYSIS OF SPRINGS WATER AND THE ROLE OF *TYPHA ANGUSTATA* AS A BIOSORBANT

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There exists a need of employing cost effective and eco-friendly materials for water treatment rather than expensive substances. The present study mainly focuses on the use of a perennial plant (*Typha angustata*) for the purification of disinfecting spring water collected from the various regions of District Muzaffarabad, Azad Jammu and Kashmir, Pakistan. The role of *Typha angustata* was evaluated by monitoring microbial, physical and chemical quality parameters. For evaluation, 25 water samples were collected from selected sites in Muzaffarabad to analyze. Majority of samples (20/25) possessed high number of microbes per Petri dish ranged from 85 to 279 with an average of 136.4. Laboratory trials were undertaken on the most promising non-modified and modified extract of *Typha angustata* (leaves). Inhibition of microbes from water samples with the non-modified extract of plant ranges from 19% to 100% with an average of 55.5% while inhibition of microbes from water samples with NaOH chemically modified extract of plant ranged from 2% to 98% with an average of 46.4%. Out of 25 samples, 10 samples were analyzed that showed the maximum growth the administration of simple and modified plant extract in water enhance the water quality by the inhibition of microbial colonies as compared to control group. Maximum efficiency was observed at 24 h, but efficiency was decreased when the incubating time was increased from 48 to 72 h. The microbial colonies were significantly increased after 24 h but after 48 and 72 h the visibility and size of microbial colonies were also increased. There is a need to carry out further studies, which include toxicity to guarantee the safety of using modified extract of *T. angustata* as a absorbant in the purification of drinking water for human consumption.

TITANIUM DIOXIDE NANOPARTICLES AS AN ANTIBACTERIAL AGENT AGAINST PATHOGENIC BACTERIA

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Microbial resistance represents a challenge for the scientific community to develop new bioactive compounds. Furthermore, resistance in microbes against antibiotics is a worldwide problem that arises due to extensive use of antibiotics. Nanotechnology holds an important area in research due to its immense use in the different fields. The smaller size, larger surface area, orientation, and physical properties of nanoparticles make them appropriate to be used for research purpose. The present study was designed to determine the antimicrobial activity of Titanium dioxide (TiO₂) nanoparticles against different clinical isolates including: *Escherichia coli*,

Pseudomonas aeruginosa, *Klebsiella pneumoniae* and *Staphylococcus aureus*, *Bacillus subtilis* bacterial strains. Minimum inhibitory concentration (MIC) was evaluated by the standard agar dilution method. The antioxidant activity and cytotoxicity of TiO₂ nanoparticles was also analysed by α - α -diphenyl- β -picrylhydrazyl (DPPH) scavenging activity and Brine shrimp assay respectively. The collected strains were cultured on different selective and enriched media and identified by physical and morphological characteristics. Six different concentrations of TiO₂ nanoparticles were used i.e. 0.2 mg/ ml, 0.4 mg/ ml, 0.6 mg/ ml, 0.8 mg/ ml, 1.0 mg/ ml and 1.2 mg/ ml to evaluate the antimicrobial activity. The MIC of TiO₂ for *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *B. subtilis* was calculated as 0.04 mg/ ml, 0.08 mg/ ml and 0.07 mg/ ml, 0.05 mg/ ml and 0.09 mg/ ml respectively. The maximum zones of inhibition (20mm) were formed at 1.2mg/ml concentration of TiO₂ against *Staphylococcus aureus*. Analysis of results showed that antimicrobial activity, antioxidant activity and cytotoxicity of TiO₂ nanoparticles increased by increasing the concentration these nanoparticles.

COMPARISON OF ANTIMICROBIAL ACTION OF PROPOLIS COLLECTED IN SUMMER AND WINTER

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Propolis is a natural product collected from honey bee hive, which bees produce through mixing plant resins, pollens and their enzymes. The current study was designed to investigate and compare the antibacterial potential and antimicrobial mechanism of action of Propolis collected in summer and winter against the peptic ulcer related pathogens. Agar well diffusion method, broth dilution and autography were used for determination of antimicrobial activity. Mode of Action was evaluated through (a) Determination of cell lysing property and (b) Scanning Electron Microscopy (SEM). Results indicate a significant antibacterial activity of Propolis against all bacterial strains tested, prominent zones of inhibition were formed. MIC and Thin layer Chromatography further confirms this activity. Investigations on mode of action indicate that Propolis performs its action through cell lysing. It was evaluated that propolis collected in summer shows better results than propolis collected in winter.

ANTIMICROBIAL PROPERTIES OF DALBERGIA, BRASSICA AND TRIFOLIUM HONEY AGAINST BURN MICROORGANISMS

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The research aimed at assessing the antimicrobial properties of *Dalbergia*, *Brassica* and *Trifolium* honey against microorganisms isolated from infected burnt skin of patients of Children's Hospital. These microorganisms were identified as *P. aeruginosa*, *E.coli*, *K. pneumoniae* and *S. aureus*. The original bacterial inoculum was serially diluted (adjusted to 1.5×10^6 CFU) and then spread onto the nutrient agar plates. Whatmann filter paper discs were soaked in three different concentrations (50%, 70%, 90%) of each of the three unifloral honeys for 48 hours and then applied onto the plates seeded with the bacteria. Solitary and synergistic effect of antibiotics (Ciprofloxacin, Imipenem, Ceftriaxone, Amikacin, and Vancomycin) with each of the three honeys was also studied. All the bacteria showed resistance to Ciprofloxacin and Ceftriaxone but none towards any of the three honeys. *Brassica* honey was the most effective at all concentrations with maximum zone of inhibition of 11.13 mm at 90% against *P. aeruginosa* followed by *Trifolium* honey with the maximum zone of inhibition of 10.75 mm at 90% against *P. aeruginosa*. The least effective was *Dalbergia* honey with maximum zone of inhibition of 10.25 mm at 90% against *S. aureus*. Antibiotic and Honey combination produced zone of inhibition of 51.5 mm much larger than when both antibiotic and honey disc were applied separately. Honey, whether used individually or in combination with antibiotic, was effective against all the bacterial isolates used in the study.

BACTERIAL SYNTHESIS OF SILVER NANOPARTICLES - THEIR CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY

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Biosynthesis of metal nanoparticles is an expanding research area due to its potential applications. The present study focuses on biosynthesis of silver nanoparticles employing bacteria and optimizing conditions to enhance mono dispersed silver nanoparticles. Initially ten bacterial isolates were screened for synthesis. Among them *Bacillus mojavensis* BTCB20 isolate was selected which had the potential of synthesizing nanoparticles of size 105 nm initially. The isolate was characterized by FTIR, XRD and AFM. Under optimum conditions such as temperature (55°C), pH (8), surfactants (Tween 20) and metal ions (K₂SO₄) the size of AgNPs was reduced from 105 nm to 2.3 nm. Molecular identification revealed 98% similarity with *Bacillus mojavensis*. Significant antibacterial activity as compared to streptomycin, against the highly resistant Gram positive and Gram negative pathogens. Conclusively, under optimized conditions *Bacillus mojavensis* BTCB20 was able to produce well mono dispersed 2.3 nm sized AgNPs and could have great potential in different applications.

PREVALENCE AND ANTIBIOTIC RESISTANCE OF STAPHYLOCOCCUS AUREUS IN SELECTED AVIAN SPECIES IN PATTOKI, PUNJAB, PAKISTAN

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Birds are susceptible to spread various pathogens to other animal's species and human being too. The present study was undertaken to estimate the prevalence, isolation and identification of *Staphylococcus aureus* and their antibiotic sensitivity in avian species inhibited at UVAS Wildlife Breeding Centre, Pattoki, Punjab, Pakistan. Fecal samples (N = 90) of various avian species (10 blue rock pigeon, 10 pea fowl, 10 ducks, 10 doves, 10 parakeets, 10 cockatoos, 10 ostrich, 10 turkeys, 10 ring necked pheasant) from A-block and C-block of UVAS Ravi Campus, Pattoki were collected and transported to Wildlife Epidemiology and Microbiology Laboratory, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Pattoki, for isolation and identification of *Staphylococcus aureus* and their antibiotic sensitivity (N = 9). For the isolation of bacteria, samples (Fecal) were cultured on Mannitol salt agar. 13 isolates were confirmed as *Staphylococcus aureus* out of 90 fecal samples. All birds' species were culture positive for *Staphylococcus aureus* except Turkeys and Cockatoos. Similarly, all *Staphylococcus aureus* isolates were resistant against thirteen antibiotics. This study provide in depth information on the prevalence and antibiotic resistance of *Staphylococcus aureus* in Pakistani birds that will be help to devise effective control strategy for cross transmission to human population.

LACTOBACILLUS BREVIS MF179529 MAY HELP IN PROTECTION AGAINST LISTERIOSIS

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Listeria monocytogenes is an opportunistic foodborne pathogen that causes human *Listeriosis* and causes high mortality particularly in immunocompromised individuals. Pregnant women are more prone to *L. monocytogenes* infection resulting in abortions. In the present study antilisterial activity of *Lactobacillus brevis* MF179529, a probiotic strain of bacteria, was investigated using murine model. Initially a pilot study was conducted to determine the dose of *L. monocytogenes* required to cause symptomatic listeriosis. In main trial,

mice were divided into 4 groups. Group I was kept as negative control, Group II was exposed to *L. monocytogenes* and maintained as positive control. Group III was fed with probiotic only while Group IV received probiotic and infection. Probiotic treatment started three days prior to infection with *L. monocytogenes*. *L. monocytogenes* ATCC19115 and *L. brevis* MF179529 were given to respective groups at a dose of 2×10^9 cfu/ml by intragastric feeding tube. Progress of infection was monitored for 7 days post infection. Animals of group IV displayed very low severity index of infection. *L. brevis* supplementation resulted in significantly less propagation of *L. monocytogenes* in liver, spleen and intestine. Positive effects were shown by *L. brevis* MF179529 consumption on the intestinal microbial equilibrium. It significantly increased number of lactic acid bacteria and reduced the total plate count, anaerobic count and *E. coli* population. These results suggest that orally administered *L. brevis* ameliorated *L. monocytogenes* infection. Safety evaluation of *L. brevis* was performed by comparing physical appearance, hematological and serological picture of *L. brevis* treated and control group (group IV and I). No significant difference in feed intake, body temperature, body index, body weight and blood picture could be detected in *L. brevis* supplemented and control groups. All these data indicate that *L. brevis* could be used for prophylactic measure and is safe for organisms.

ANTAGONISTIC ACTIVITY OF *PSEUDOMONAS FLUORESCENS* AGAINST FUNGAL PLANT PATHOGEN *ASPERGILLUS NIGER*

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Soil-borne plant pathogenic fungi are of a major alarmed problem in agriculture. A variety of fungi are well-known to cause important plant diseases, resulting in a significant loss in agricultural crops. A biological control mechanism is stated alternative to synthetic chemicals using microbial antagonists for controlling plant diseases. Biocontrol methods are harmless, cost effective and eco-friendly. Bacteria are able to produce a wide range of metabolites with fungicidal capabilities. The present study was performed to figure out the antagonistic activity of *Pseudomonas fluorescens* against fungal plant pathogen *Aspergillus niger* (black rot of onions, crown rot of peanuts). *Pseudomonas fluorescens* was isolated from rhizospheric soil of healthy plants. King's B medium was used for the isolation of *Pseudomonas fluorescens*. Seventeen isolates of *Pseudomonas fluorescens* were isolated from thirty soil samples. All isolates produced positive results of biochemical tests (catalase, oxidase, starch hydrolysis, gelatin liquefaction). *Aspergillus niger* was isolated from diseased onion and it was chosen because it is commonly occurring and fast growing fungus. Antifungal assays (cross streak assay, pour plate method, agar well diffusion method) were done to check the antagonistic activity and zone of inhibition was observed. Clear zone around bacterial growth was regarded as indicator of inhibition of fungus growth measured in mm and % inhibition of fungal growth was measured. Results showed significant antagonistic potential of *P. fluorescens* against *A. niger* with reduction of 65.1 - 81.3 % in fungal colony diameter in cross streak assay, 63.9 - 70.9 % in pour plate assay and 71.84 ± 1.56 in agar well diffusion method. Seeds were also treated with better performing isolate of *Pseudomonas fluorescens* and test fungus to study antagonism and observed 100 % of growth index when treated with Pf4 strain and showed 50 % inhibition of disease. *In-vitro* assays revealed these isolates were antagonistic in nature and showed significant antifungal activity against *Aspergillus niger* with different levels of inhibition pattern.

PREVALENCE AND SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACILLI FROM CLINICAL SAMPLES IN ISLAMABAD AND RAWALPINDI

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Gram negative bacterial infections execute massive load on medical structures because of great incidence of community acquired and nosocomial infections. Occurrence and prompt blowout of resistance producing

enzymes in *Enterobacteriaceae*, *Pseudomonas* and also in *Acinetobacter* species is becoming a most important communal healthiness disaster globally, as well as accountable for huge extent of infections. Objective was to combat this evolving resistance a constant investigation of antibiotics vulnerability design of gram negative bacteria is vital at local levels that can then afford support in management of operational preliminary treatments. Clinical isolates of gram negative *Bacilli* from such patient samples as blood, breast milk, cerebrospinal fluid (CSF), ear swabs, pus, semen, sputum, stool, throat swab and urine was carried out. The data was analyzed in terms of patient demographics, sample origin. The data was analyzed using Chi-square (χ^2) test, confidence interval (CI) and odds ratio analysis for paired samples. The multiple antibiotic resistance index (MARI) of different antibiotics against isolated gram negative bacteria was calculated. A total of 1522 clinical samples were included in this study. Out of which 341 were identified as gram negative bacteria. The overall prevalence of bacterial infections caused by gram negative bacteria was predominantly observed in clinical samples obtained from female patients. The highest prevalence rate of gram negative bacterial infections was within the age group 41-65 years. *Enterobacteriaceae* were isolated in 252 samples, whereas *Non Enterobacteriaceae* was isolated from 58 samples. A difference in the antibiotic susceptibility profiles of *Enterobacteriaceae* and *Non-Enterobacteriaceae* was observed. Present study provides a valuable data to compare and monitor the status of antibiotic resistance among gram negative bacterial pathogens to improve the empirical treatment in Rawalpindi and Islamabad. Further research as the constant investigation of antibiotics vulnerability design of gram negative bacteria is vital at different localities that can then afford support in management of operational preliminary treatments.

SCREENING OF LACTIC ACID BACTERIA FOR BACTERIOCIN PRODUCTION FROM FRUITS AND VEGETABLES IN DISTRICT MARDAN

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The current research work was designed to evaluate the bacteriocin activity of Lactic Acid Bacteria (LAB) isolated from fresh fruits and vegetables against selected pathogenic microorganisms. A selective medium (MRS) was used for the isolation of LAB. The antagonistic properties of lactic acid bacteria were screened against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* by using well diffusion method. The strongest antibacterial activity was exerted by bacteriocin producer strain Cb12 against *Staphylococcus aureus* with a Zone of Inhibition 20.5mm. M1 and A7 strains did not show inhibitory activity against *Salmonella typhi*, While A9 and Cm4 showed no inhibitory activity against *Pseudomonas aeruginosa*. These bacteriocin producers were identified morphologically and biochemically by standard methods as *Leuconostoc*, *Lactobacillus* and *Pediococcus* species. The bacteriocins extracted from these lactic acid bacteria retained its antibacterial activity after treating with temperatures 37°C and 45°C but showed a little decrease in activity at 60°C and 80°C. Similarly, bacteriocinogenic lactic acid bacteria retained their activity at different pH values 4, 6 and 8. It was concluded that fruits and vegetables have Lactic Acid Bacteria (LAB) which exhibit significant bacteriocin activity against both Gram positive and Gram negative harmful bacteria. It is recommended that these effective isolates should be used for the preservation of various food stuffs.

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERN AGAINST GRAM POSITIVE BACTERIAL PATHOGENS IN ISLAMABAD AND RAWALPINDI

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Antibiotic resistance in gram-positive bacteria is a persistent problem. Both infection control and antibiotic selective pressure are important factors in its spread. The appearance of antibiotic resistance has been

a problem day by day because of overusing antibiotics. It is desirable to be conscious of this problem to elude inappropriate use, repeated misuse, insufficient dosages, easy accessibility of antibiotics and must be planned the treatment schedule after proper laboratory exploration. To warfare this evolving resistance a constant investigation of antibiotics vulnerability design of gram positive bacteria is vital at local levels that can then afford support in management of operational preliminary treatments. Clinical isolates of gram positive bacteria from such patient samples as blood, breast milk, cerebrospinal fluid (CSF), ear swabs, pus, semen, sputum, stool, throat swab and urine was carried out. The data was analyzed in terms of patient demographics, sample origin. The data was analyzed using Chi-square (χ^2) test, confidence interval (CI) and odds ratio analysis for paired samples. The multiple antibiotic resistance index (MARI) of different antibiotics against isolated gram positive bacteria was calculated. A total of 1522 clinical samples were included in this study. Out of which 155 were identified as gram positive bacteria. The overall prevalence of bacterial infections caused by gram positive bacteria was predominantly observed in clinical samples obtained from male patients. *Staphylococcus* species were isolated in 110 samples, whereas *Enterococcus* species was isolated from 25 samples and *Streptococcus* species was isolated from 6 samples. A difference in the antibiotic susceptibility profiles of *Staphylococcus* species, *Enterococcus* species and *Streptococcus* species was observed.

USE OF PROPOLIS AGAINST SELECTED MICROBES INCLUDING HONEY BEE PATHOGENIC FUNGI *ASCOSPHAERA APIS*

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Propolis is plant resinous substance collected by honey bees. It is one of the most fascinating bee hive product not only used structurally to fill out cracks in the bee hive but it also has several diverse bioactivities and is used against pathogenic microbes, suggesting its potential interesting antimicrobial properties. Since reports dealing with the activity of Pakistani propolis against selected pathogenic microbes including honey bee pathogenic fungi (*Ascosphaera apis*) are not available. In this study, crude propolis and ethanolic extracts of propolis (EEP) were used. The methodology employed was agar diffusion method using different culture media. Antimicrobial activity was determined by zone of inhibition in diameters (mm) after 24 to 48 hours of incubation at 25° to 37°C. Results demonstrated that EEP significantly inhibited the growth of human pathogenic fungi *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Candida krusei* including infectious honey bee fungal pathogen (*Ascosphaera apis*) Interestingly, the crude propolis was not active against the tested strain of fungi and also EEP inhibited the growth of *Serratia marcescens* and *Salmonella typhi* while crude propolis had no significant effect on the growth of both pathogen *Serratia marcescens* and *Salmonella typhi*. The study concluded that Pakistani propolis have novel compounds that can be use against various microbial pathogen.

ANTIMICROBIAL ACTIVITY OF THREE ESSENTIAL OILS AGAINST GRAM POSITIVE BACTERIA

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Essential oils of plants and herbs have been practiced since ancient times for therapeutic purposes, flavoring and preservative agent or as an alternative to medicines. Essential oils also known as the volatile oils extracted from the different parts of the plants such as bark, flowers, buds, leaves, fruits, seeds, wood and roots through many techniques of extraction. Higher and aromatics plants usually used in folk medicine. Maximum of their properties are owing to essential oils created by their secondary metabolism. These essential oils used in food preservation, pharmaceuticals, alternative medicine and natural therapies. Essential oils and extracts are

able to control microorganisms related to skin, dental caries, and food spoilage, including Gram-negative and Gram-positive bacteria and so many others with varying degree of effectiveness. In present days to minimize or cope with the alarming situation caused by the bacterial resistance the need to develop potential natural antimicrobials has increased to use as a substitute. The present study was thus directed to assess the antibacterial prospective of three essential oils including caraway (*Carum carvi*), star anise (*Illicium veraum*) and pepper mint (*Mentha piperita*). For this purpose, ten raw milk samples were collected from different animals (buffalos) and bacterial samples were isolated. *Staphylococcus aureus* isolates were identified through morphological identifications and Biochemical tests (catalase test, nitrate reduction test, coagulase test, mannitol salt agar test and blood agar plate test). Antimicrobial activity checked by different concentrations of three essential oils (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128) in Agar well diffusion method. All the essential oils showed inhibitory effect at varying levels. In agar well method maximum zone of inhibition observed against *Staphylococcus aureus* S5 isolate with mean of inhibition zone diameter observed as 12.0 ± 0.3 mm of peppermint oil while minimum 2.4 ± 0.3 mm observed in S2 of star anise essential oil. Peppermint oil (*Mentha piperita*) showed the highest activity. The order of action potential of essential oils observed as *Mentha piperita* higher than *Carum carvi* and *Carum carvi* higher than *Illicium varum*. ANOVA was applied to all values and P values less than 0.05 considered as significant. The overall significant result observed by agar well diffusion assay with extreme action of essential oils recorded at 1/2, 1/4 and 1/8 dilutions.

BACTERIAL INOCULATION AFFECTS INTERNAL ANATOMY OF *TRITICUM AESTIVUM*

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Plant growth promoting rhizospheric bacteria (PGPR) have been found to affect the plants beneficially. These rhizospheric bacteria use different mechanisms to trigger the growth of the plants. In the current study, auxin-producing *Cronobacter* and *Enterobacter* spp. were used to treat seeds of *Triticum aestivum* var. FD-08 and the inoculated and non-inoculated plants were studied to investigate the impact of bacterial treatment on plant growth. For this purpose, growth and biochemical parameters as well as anatomical parameters of treated and non-treated plants were studied. It was observed that plants treated with bacterial strains exhibited pronounced increase in different growth parameters. Biochemical analysis of inoculated plants has also shown increase in protein, auxin, chlorophyll "a", chlorophyll "b" and total chlorophyll contents of inoculated plants as compared to the non-inoculated control plants. Root and stem anatomy of the treated and non-treated seedlings were also studied. Microscopic observations revealed that the selected auxin-producing bacterial strains improved the anatomical features of roots and stem of the inoculated seedlings as compared to the non-inoculated seedlings. Thus, these auxin-producing bacterial strains can be used as biofertilizers to enhance crop yield.

IN VITRO BIOLOGICAL CHARACTERISTICS OF PROBIOTICS LACTOBACILLUS STRAINS SCREENED FROM CHICKEN GUT AND THEIR ROLE IN THE PREVENTION OF PATHOGENS COLONIZATION

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An experiment was conducted to evaluate probiotic properties of some *Lactobacillus* strains isolated from intestine of the adult chickens. All the strains were isolated from ileum, caecum and colon of healthy chickens. These were washed with sterilized PBS, inoculated on MRS agar plates and screened. Sequence analysis of 16S r RNA revealed isolates of *L. crispatus* (strains 5-1-1, 5-3-2 and N-11), *L. johnsonii* (6-3 and N-

23), *L. salivarius* (N-7 and N-14) and *L. saermneri* (M-11). Resistance level varied significantly ($p < 0.05$) among all the strains. All the isolates except *L. saermneri* had the highest sensitivity to antibiotics such as kanamycin, ampicillin, streptomycin and gentamycin. Significantly ($p < 0.05$) lower effect of LAB was determined on growth during the experiment. It can be concluded from the present study that *L. crispatus*, *L. johnsonii* and *L. salivarius* have antimicrobial activity and antibiotic susceptibility properties and can be used in broiler chicken as probiotic.

5. MOLECULAR BIOLOGY

DEVELOPMENT OF ANTIBODIES AGAINST HEPATITIS C VIRUS ENVELOPE GLYCOPROTEINS E1 AND E2

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HCV infection is a critical global issue. Currently no vaccine is available to prevent HCV infection due to divergence of nucleotide sequence of various strains. There is a strong need to develop vaccine against HCV infection because the current therapy is long, costly and has significant side effects. The current study is based on the utilization of envelope glycoproteins E1 and E2 for the development of recombinant vaccine against HCV. It encompasses the amplification, cloning, expression, purification and antibodies production of HCV3a structural genes E1 and E2. Blood samples from the patients infected with HCV 3a from local region were collected. Total RNA was extracted and cDNA was synthesized through random hexamers. Sense and antisense primers for the amplification of the structural envelope glycoproteins genes E1 and E2 were designed by using Primer 3 software. Nested Polymerase Chain Reaction was employed for the amplification of E1 and E2 genes. Sequencing was performed and the amplified genes were confirmed as HCV3a envelope glycoproteins. Cloning was performed by using pTG19, a cloning vector. The structural genes were sub-cloned separately in pET21a expression vector. These were used to transform in *E. coli* BL21 (DE3), an expression host and structural HCV proteins were expressed in the bacterial system. The E1 protein was purified partially by salting out through ammonium sulphate precipitation and by anion exchange FPLC technique. The E2 protein was purified by repeated sonications, denaturation and refolding procedure through fractional dialysis with urea. It was further dialyzed to get rid of any salt present in the protein. The purified proteins HCV3a E1 and E2 were confirmed through ELISA with HCV 3a infected human sera. The purified proteins were injected into rabbits to raise antibodies. The antibodies produced were confirmed through ELISA using secondary anti rabbit antibodies. The rabbit antibodies produced against E1 and E2 proteins used as antigens were highly specific. In short, these purified proteins have antigenic activity and can be investigated further to be used as vaccine against hepatitis C.

COST-BENEFIT ANALYSIS OF GENETIC AND GENOMIC DIAGNOSTIC TESTING

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The development of next-generation sequencing technologies has led to the clinical application of diagnostic tests that question the whole genome, providing opportunities for the diagnosis of rare hereditary diseases or providing information for therapeutic targeting. The new genomic diagnostic tests compete with traditional diagnostic methods, including genetic testing of individual genes and other clinical strategies for limited health budgets. In this case, a cost-benefit analysis based on a decision analysis model is a useful way to help assess the costs and consequences of introducing a new medical intervention. This perspective raises key methodological, technical, practical, and organizational challenges that policy makers in charge of allocating healthcare resources must consider meeting the challenges of being relatively cost-effective and robust in time for a growing number of emerging genomic tests Information.

MOLECULAR CHARACTERIZATION OF A CILIATE (*PARAMECIUM* SP.) ISOLATED FROM DIFFERENT INDUSTRIAL WASTE WATERS

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Anthropogenic activities add toxic substances like heavy metals in the aquatic ecosystem that may cause

hazardous effects to both animals and plants. In this context bioremediation is one of the best adaptations to clean the environment. Waste water samples were collected from ponds receiving effluents from the industries located in Shah Pur Kanjra and Kot Lakh Pat in Lahore. A multiple heavy metal resistant ciliate *Paramecium multimicronucleatum* was isolated and characterized with the help of 18SrRNA and histone H4 biomarkers. The optimum temperature and pH were found to be 22°C and 7.5pH. Growth patterns of *P. multimicronucleatum* was observed with and without metal stress. The metal ions slowed down the growth of *Paramecium* sp. as compared to the culture grown without metal stress. The minimum inhibitory concentration of cadmium, zinc, mercury and lead were 50µM, 800µM, 120µM and 700µM respectively. Cadmium uptake was determined by atomic absorption spectrophotometry which concludes that initially *Paramecium* sp. showed resistance even at 10µM and maximum uptake of metal at day 7 that was 0.04µg/ml was recorded. Maximum uptake of metal was observed at 20µM, 30µM and 40µM at day 15, 16 and 19 was 0.32µg/ml, 0.34µg/ml and 0.25µg/ml respectively.

PROTEOMIC AND GENOMIC ANALYSES OF BORON-TOLERANT BACTERIA

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Boron (B) is an essential micronutrient for plants and possibly for animals. On the other hand, it is also toxic to living cells when present above a certain threshold. Because of its toxic effects at high concentrations for micro-organisms, it is reported to be used as a food preservative, as well as insecticide especially against cockroaches. However, only recently many species of bacteria (such as *Bacillus boroniphilus*; *Bacillus firmus* strain KC; *Algoriphagus boritolerans*; *Gracilibacillus boracitolerans*; *G. pakistanensis*; *Lysinibacillus boronitolerans*; *L. parviboronicapiens*, *L. pakistanensis*; *Variovorax boronicummulens*; *Rhodococcus baikonurensis* and *Brevibacterium pakistanensis*) have been reported to tolerate very high concentration of boron, which is toxic to most of the living cells. Interestingly, a bacterial species *Bacillus boroniphilus* exhibited a property of not only tolerating very toxic concentration of B but also require B for its growth. This unique property of *B. boroniphilus* offers a great deal in understanding the biochemistry of B; and to answer the question “why boron is needed in any living cells?”. Previously, boron has been reported to have a role in quorum sensing in bacteria. Other established physiological role of B include the functioning of various enzymes, and other proteins of the plasma membrane, transport processes across the membrane and membrane integrity. In plants, the physiological function of B is to form esters with a *cis*-diol moiety in rhamnogalacturonan-II (RG-II) that is required for stabilization and integrity of cell wall. There could be other putative B binding biomolecules; but it is yet hard to decide the major role of these ligands besides RG-II. To avoid both B deficiency and toxicity problems, it is important to maintain optimum B in plants cells through regulating transport processes. Identification of several B transporters, BORs, in *Arabidopsis* and rice helped in better understanding of B transport and homeostasis at molecular and biochemical level. However, physiological mechanism of boron tolerance in bacteria suggested a very strong efflux transport of boron across the membrane. This prokaryotic efflux B transport system is assumed to be much more stronger than in eukaryotic cells. Molecular identification of these system will help to deepen our understanding in boron biochemistry of living cells. Whole genome analyses in prokaryotes have become a powerful tool for gaining insight into molecular and functional biology of any organism, especially in prokaryotic unicellular organism. Once genome sequences are determined, an immediate task is to identify all the available genes to reveal evolutionary relationships and to predict their functions. Fortunately, most of the bacterial proteins are highly, or at least moderately, conserved in evolution. The functions of any uncharacterized proteins can be experimentally determined through proteomics and metabolomics approaches, and the accuracy of homology-

based predictions can be critically evaluated. In membranes of Prokaryote, glycoproteins and glycolipids are good candidate ligands for possible B functions. To identify novel genes and biomolecules involved in biochemistry of boron, we have sequenced the whole genome of eleven boron tolerant species of bacteria. Genome dissection of these B-requiring and highly B-tolerant strains will provide enormous information that can be applied to functional biology. Further studies were conducted by gel free proteomic analysis of boron tolerant bacteria to identify the possible proteins involved in boron transport across the cells. In our presentation, we will provide insight into the proteomic and genome comparison of boron tolerant bacteria and novel gene(s) to predict their functional role in boron biochemistry.

KNOCKDOWN OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDE RECEPTOR IN *AGROTIS IPSILON* (HUFNAGEL) (LEPIDOPTERA: NOCTUIDAE)

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RNA interference (RNAi) holds great potential as a new measure for pest management. However, RNAi effectiveness varies greatly in insects from species to species. Insects in the order Lepidoptera are among the most difficult species for utilization of RNAi-based methods. In present study, we chose the black cutworm, *A. ipsilon* as a target for optimizing conditions for RNAi-based gene silencing. The siRNA and dsRNA to knockdown the PBANR involved in the production of sex pheromone in adult *A. ipsilon* females were used. We injected 20µl of siRNA, dsRNA, dsGFP, and water into the 8th and 9th abdomen segment of an adult *A. ipsilon* female and evaluated its expression 72 hrs after injection. The results of qRT-PCR demonstrated that the mRNA levels were significantly reduced more than 90% in PBANR siRNA-treated *A. ipsilon*, however, 56% knocked down by dsRNA, respectively than in dsGFP, water and non-injected controls.

CLONING OF THE PULLULANASE GENE OF *THERMUS AQUATICUS* DSM 625 AND EXPRESSION OF THE ENZYME IN *ESCHERICHIA COLI*

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The present study deals with gene cloning, expression and purification of a thermostable pullulanase from *Thermus aquaticus* (DSM 625). The pullulanase gene was isolated and then purified from the thermophile and cloned successfully using the cloning vector pTZ57R/T. The 670 bp gene was expressed in *E. coli* DH5α host cells. The gene was expressed in the expression vector pET-21a (+) and expression host cells BL21 Codon Plus. Sequencing analysis confirmed nucleotide sequence of the cloned gene and phylogenetic analysis showed homology among many organisms. The enzyme was purified by using different techniques. The ammonium sulfate precipitation technique was used for the purification of pullulanase enzyme from cell lysate. No activity was observed in 20-40% saturation level. The recombinant enzyme had highest specific activity of 66.0 U/mg at 70-80% fraction. The purified 25 kDa enzyme was further characterized through various parameters. Enzyme assays were carried out to determine physical characteristics. Various metal ions were used to determine the

effect on pullulanase activity. The Pb⁺² ions had no effect but Ca⁺² and Zn⁺² somewhat reduced the activity. On the other hand, Hg⁺² decreased it to 89% which is significant. Mg⁺² and Cd⁺²⁴ both reduced the enzyme activity to more than half and SDS halted it up to 73%. The thermostability of the enzyme was analysed on a wide temperature range (20-100 °C). It was determined that peak activity of the enzyme was at 80 °C with pH 6.0. Further optimization and investigation is required to benefit from the thermostable properties of this enzyme to fulfil potential biotechnological prospects.

CLONING AND EXPRESSION OF CADMIUM RESISTANT GENE FROM *ANOXYBACILLUS* SP

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Cadmium is one the heavy metal found in nature that is toxic for most of the living organisms especially humans. As a result of its use in industries, cadmium is released from industries along with the industrial waste water. Cadmium is found to cause serious health issues in mammals including cancer. So a need for its removal from the industrial waste water is required to stop it from becoming a part of the natural ecosystem related to animal or human consumption. For this purpose, it is found that few bacterial cells have the ability to survive in high heavy metal concentration. This is due to the presence of specific gene in their DNA. Similar property is exhibited by *Anoxybacillus* sp. CadC gene is present in the genome of *Anoxybacillus* sp which was checked from the whole genome sequence of this species present at NCBI (a database). Designing specific primers for CadC gene and amplifying this gene in a thermocycler is followed by the cutting of this specific DNA fragment by the restriction enzyme whose restriction site was added in the primer designed. After selecting an appropriate vector, in this case pTZ57R/T vector was used. Preparation of competent cells of *E. coli* DH5α and their incubation with the vector was done to produce clones of bacterial cells that possess the desired DNA fragment. The positive control were selected, that is the white colonies. These are those *E. coli* cells that possess the CadC gene from *Anoxybacillus* sp. These *E. coli* cells can now be used for the treatment of industrial waste water and hence bioremediation.

DNA BARCODING OF CYPRINID FISHES (*LABEO ROHITA*, *CATLA CATLA* AND *CIRRHINUS MRIGALA*)

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The mitochondrial Cytochrome Oxidase 1(CO1) gene is used as standardized, reliable and testimonial certification as genetic marker for a universal species-level bio-cataloging system compared with the morphological identification. The Barcoding (CO1) of freshwater fish species of family Cyprinidae (*Catla catla*, *Cirrhinus mrigala*, and *Labeo rohita*) was conducted in present study. Amplified CO1 gene through PCR was sequenced and analyzed by means of bio-informatics software. Conspecific, congenric K2P nucleotide divergence and composition, number of haplotypes and haplotype diversity was designed and calculated. From these findings, it was concluded that the gene sequence, CO1 not only serve as milestone for the identification of related species at molecular level but also distinguishing and estimate the nucleotide deviation among species, genera, and families. The ability to assign species with high exactitude from DNA samples of different quality and origin has foremost effectiveness in the field of fisheries, its conservation and fish products genuineness and sustainability.

DNA BARCODING OF TOR SPECIES OF RIVER POONCH (AZAD JAMMU AND KASHMIR) AND ATTOCK HATCHERY, PAKISTAN

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DNA bar-coding is a taxonomic method, uses small genetic markers in organism's mitochondrial DNA (mtDNA) for identification of particular species. It uses sequence of mitochondrial cytochrome c oxidase subunit 1 (COI) gene as a tool for species identification. DNA barcoding is more accurate and reliable method as compared to morphological identification. Complete COI gene was amplified using PCR and sequenced from samples collected from Poonch Rivers of Azad Jammu and Kashmir and Attock hatchery, Pakistan. Identification of species was done through morphology, BOLD (99.6%) and NCBI (99.7 %) reference sequences of tor species. The overall base composition of Tor samples were 29.46% of T, 25.73% of C, 27.27% of A, and 17.54% of G, A+T content 56.74% and G+C content 43.26% showing anti-G bias. The Ts/Tv bias (R) was 2.51. Multiple alignments of COI mtDNA gene resulted in a range of 1551 base pairs. Out of 1551 consensus sites, 1490 were constant, 61 characters were variable, in which 54 were parsimony informative, and 7 variables were parsimony uninformative. Our results (DNA sequences) established that Tor species of Attock hatchery and river Poonch was be similar with NCBI *Tor putitora* sequences and not with *Tor macrolepus*.

EFFECT OF CADMIUM CONCENTRATION ON METALLOTHIONEIN CONTENTS IN SHELLFISH SPECIES

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Cadmium accumulation and metallothionein contents were investigated in three shellfish (*Scylla serrata*, *Portunus reticulatus* and *Penaeus merguensis*) species through bioassay. The effect and accumulation pattern of Cd were observed in different body parts (muscles, gills and carapace) through exposure of various Cd (0.5 ppm, 3 ppm and 5 ppm) concentrations at 3, 6 and 9 days. There are significant ($p < 0.05$) difference were observed in all the three investigated species in body parts with duration and concentrations when compared to controlled groups. In muscles of shellfish; the metallothionein contents were also observed and significant ($p < 0.05$) differences were observed between the species and duration of exposure. The high metallothionein contents (38.76 ± 0.31) was found in *S. serrata* followed by (38.374 ± 0.068) in *P. merguensis* and (35.41 ± 0.009) in *P. reticulatus*. These preliminary finding confirms that the synthesis of metallothionein's and binding capacity of these proteins are restricted and can be used as biomarkers of Cd pollution in seafood particularly shellfish (crabs and shrimps) species.

MOLECULAR EPIDEMIOLOGY OF BREAST CANCER IN PAKISTAN; DIAGNOSIS, PATHOLOGY, RISK FACTORS

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Male breast cancer is a rare malignancy and only reported for 1% of all diagnosed breast cancers. It tends to be diagnosed at later age then the breast cancer in females likely because of low awareness. Risk factors include genetic predisposition, alterations to estrogen-progesterone ratio, previous HCV history, obesity and alcoholism. Through genomic and proteomic approaches researchers have been able to provide the insight of MBC through biomarkers. This article will highlight the current situation of MBC in Pakistan, risk factors and

its molecular epidemiology. The international collaboration with Umm Al Qura University will also facilitate the future planning on proteomic approaches for early diagnostic and treatments of male breast cancer in Pakistan.

CLONING, SEQUENCING, PURIFICATION AND CHARACTERIZATION OF THERMOSTABLE ASPARTATE AMINOTRANSFERASE FROM *THERMOPAKISTANIENSIS*

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Aspartate aminotransferase gene consists of 1182bp nucleotide encodes for 393 amino acids was sequenced, cloned, expressed and purified to homogeneity. Enzyme exhibited maximum activity at 65°C at pH 7. Mass spectrometry determined molecular mass of 42562 Da and gel filtration indicated the protein exist as a dimeric form. Thermostability experiment showed 100% stability of a protein at 65°C for 16 hours and half-life of 15mins at 75°C. The thermal denaturation studies by CD spectroscopy showed no significant change in ellipticity of the helical structure of the protein below 70 °C. Km and Vmax values towards aspartate were 1.61 mM and 97 µmol min⁻¹ mg⁻¹ and towards α-ketoglutarate were 2.5 mM and 50 µmol min⁻¹ mg⁻¹, respectively. Substrate specificity experiment indicated maximum activity with aspartate and its respective keto acid (α-ketoglutarate) while exhibit 27% of activity with Tyr and 16% of its activity with Pro and Cys and no activity with Glu how ever in reverse reaction of Glutamate with its keto acid, oxaloacetate, 70% of activity was observed. Neither metal ions nor EDTA exhibited a significant effect on the enzyme activity indicating that the enzyme activity of AST-SBS is not dependent on any metal ion. Pyridoxal phosphate quantification exhibited 0.1 mole of PLP per mole of enzyme. Amino acid analysis showed high contents of charged and especially acidic residues of Aspartate and glutamate in structure of ASTSBS enzyme. Homology modeling determined the Dimeric structure of ASTSBS which contained high number of proline on a surface of each subunits as compare to its mesophilic counter parts that is a reason for stability of a protein at high temperature.

MORPHOLOGICAL DESCRIPTIONS AND MOLECULAR CONFIRMATION OF *LYPEROSOMUM (LYPEROSOMOIDES) PAKISTANENSIS* N. SP; BASED ON NUCLEAR AND MITOCHONDRIAL DNA SEQUENCES

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The use of DNA barcoding as a taxonomic tool could efficiently disclose hidden biodiversity, more rapidly and more reliably than traditional morphological methods alone. Partial or complete one, two or more genes (DNA barcoding) are able for trematodes diagnosis and systematics. *Lyperosomum (Lyperosomoides) pakistanensis* n. sp (Digenea: Dicrocoeliidae) was described in the previous study from the bile ducts of *Corvus splendens* collected from district Dir Lower, KP, Pakistan. The species was described as new, based on morphological description including general body shape and size; shape, size and position of different organs and structures. To confirm the species identity, the permanently mounted slides were brought to the State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute (LVRI), Chinese Academy of Agricultural Sciences (CAAS) for further molecular studies. Two specimens were removed from Canada balsam mounted slides with the aid of xylene. The total genomic DNA (gDNA) was extracted for the amplification and sequencing of nuclear ribosomal DNA (rDNA) and mitochondrial DNA. Partial or nearly complete sequences of the large subunit rDNA (28S) with internal transcribed spacers 1 (ITS1) of the nuclear

rDNA, the partial CytB (406 bp), Nad1 (530 bp) and Cox1 (444 bp) from mitochondrial genome were determined and annotated. Sequence comparison of the 28S rDNA(1269bp) revealed 96% similarities with available sequences of *Brachydistomum ventricosum* (accession numbers KU563713.1). The phylogenetic analysis of the present species with other dicrocoelidestrematodes based on nucleotide sequences of partial 28S, Nad1 and Cox1, using neighbor joining and maximum likelihood methods (in MEGA7), revealed that the present species is well separated from *Lyperosomum* but closely related to *Brachydistomum*. However, except the presence of tapered and short anterior body, all other morphological descriptions supported that this species should be placed in the subgenus *Lyperosomoides* of the genus *Lyperosomum*. In conclusion, the identity of *Lyperosomum (Lyperosomoides) pakistanensis* n. sp. was supported by nuclear and mitochondrial DNA sequences.

GENETIC DIVERSITY OF THE *PORTUNUS SANGUINOLENTUS* (HERBST, 1783) (DECAPODA, BRACHYURA, PORTUNIDAE) IN INDO WEST PACIFIC REGION BASED ON MITOCHONDRIAL DNA 16S rRNA GENE

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The three-spot swimming crab *Portunus sanguinolentus* (Herbst, 1783) is a commercially important species and widely distributed in the Indo-Pacific region. During the present study, the genetic diversity of *Portunus sanguinolentus* was evaluated in the Indo west Pacific region (India, VietNam, China and Pakistan) by using mitochondrial non coding 16S rRNA gene sequences. In 16S rRNA the numbers of haplotype was determined a total of 6 haplotypes (*h*) was identified from 10 sequences. The haplotype diversity (*hd*) was (0.889±0.075) and significantly different ($P < 0.01$). The nucleotide diversity (*pi*) (0.00815 ± 0.00379) showed non-significant difference. Neutrality test Tajima's D was estimated ($D = -1.55773$) and was $P > 0.10$ negative and no significant deviation from mutation-drift equilibrium. Evolutionary divergence (0.042 ± 0.005) in overall sequence pairs of *P. sanguinolentus* was also estimated. The topology evaluated that *P. sanguinolentus* of Indo Pacific region were closely related to each other as supported by the bootstraps and gene flow is present.

PHYLOGENETIC ANALYSIS OF EXOTIC FISH, *OREOCHROMIS MOSSAMBICUS*, OF PAKISTAN BASED ON CYTOCHROME OXIDASE SUBUNIT 1 (COI) GENE

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Tilapia *Oreochromis mossambicus* is an exotic fish introduced in Pakistan from Malaya in 1951. Due to its omnivorous food habits and adaptability to different harsh environmental conditions such as salinity and temperatures considered as a successful species of culture in Pakistan. However, the taxonomy and phylogeny of the Tilapia fishes are poorly understood found in Pakistan. In the present study, samples of *O. mossambicus* were collected from the different aquatic system of Pakistan and investigated using Cytochrome c Oxidase subunit I (COI) of mitochondrial genes for their relationships. DNA extraction was performed from the fin tissue of collected specimen by using modified salt extraction method and Cytochrome oxidase-I (COXI) gene was amplified using universal primers for fish, and amplified products were sequenced. Sequencing results showed that specimens belonged to species *O. mossambicus*, of family Cichlidae. Sequences analysis was done by using the MEGA 7 software, and cladograms were constructed by using the neighbor-joining (NJ) method based on Kimura two-parameter (K2P). Additionally, genetic and ranked distances were derived by using the software, Automatic Barcode Gap Discovery (ABGD) and Barcode Gap Analysis (BGA). No significant

divergence and barcode gap was observed. The sequence analysis of the genes revealed that all specimens are genetically similar. The COI gene sequence not only confirms the species but also separate the populations of *O. mossambicus* from other tilapia species.

NOVEL SINGLE NUCLEOTIDE POLYMORPHISM IN THE EXON 3 OF *MYOC* GENE ENHANCE THE RISK OF GLAUCOMA

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Genetic polymorphism on *MYOC* gene alters the myocilin protein leading to the interruption in normal regulation of Intraocular pressure (IOP) that ultimately causes glaucoma. The present study was conducted with an aim to identify the polymorphism on exon 3 of *MYOC* gene in glaucoma patients of Lahore division. For this purpose a case control study was conducted with 100 patients and 100 controls subjects. DNA was extracted from each blood samples and targeted DNA fragment was amplified by PCR. Polymorphisms were identified through sequencing. It was observed that allelic and genotypic frequency of rs74315341 and rs879255525 were significantly associated with glaucoma in studied population. The polymorphism on rs74315341 polymorphic site led to the change of Arginine into Serine whereas rs879255525 transforms Lysine into Asparagine. Haplotype TGAAGCCATTTC was found to be significantly associated with disease onset whereas the haplotype GGAAGCCATTTC was found to be protective against disease development. In conclusion *MYOC* gene polymorphisms were identified as susceptible regions for glaucoma onset in population of Lahore division. Our paper is the first report to identify a novel mutation rs879255525 for glaucoma on exon 3 of *MYOC* gene.

IL-16 GENE ASSOCIATION WITH OSTEOARTHRITIS PATIENTS OF LAHORE, PAKISTAN

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Osteoarthritis (OA) is a common but complex disease characterized by the degradation of articular cartilage, often resulting in joint disability. The current study was designed with an aim to determine the relationship between *IL-16* gene and OA development in Lahore division. For this purpose a case control study was conducted on 30 patients and 30 controls. Patients and controls were age and sex matched. DNA was extracted from each blood sample and genotypes were identified by PCR-Sequencing technique. Polymorphism was identified on rs11556218 polymorphic site on *IL-16* gene. Genotype TT and allele T was significantly varied among patients and controls in studied population. The polymorphism on rs11556218, polymorphic site represented Asparagine (Asn) to Methionine (Met) substitution, leading to the dysfunction of Pro-inflammatory cytokine, IL-16, hence showing a strong relation with development of OA in studied population. In conclusion, *IL-16* gene polymorphic site rs11556218 on exon 6 was identified as a susceptible region of mutation for OA onset in population of Lahore division.

SEQUENCE ANALYSIS, MULTIPLE SEQUENCE ALIGNMENT, PHYLOGENETIC ANALYSIS AND SECONDARY STRUCTURE PREDICTION, MOLECULAR MODELING OF ZEBRA FISH FIBRONECTIN TYPE III DOMAIN CONTAINING 5 (FNDC5)/IRISIN

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Irisin is a myokine that regulates and improves the expenditure of energy by triggering the white adipose tissue to 'brown' (Perakakis N et al, 2017). Irisin is a muscle-secreted protein was discovered since 2012 (Gouveia MC et al, 2016). Irisin hormone is produced by the breakdown of Fibronectin type III domain-containing protein 5, which is determined by the FNDC5 gene (Erickson HP, 2013). The FNDC5 gene produces a prohormone, a single-pass type I membrane protein (212 amino acids in humans and 209 in mice and rats) that is kept synchronized through exercising muscles and forms irisin by undergoing post-translational processing (Boström P et al, 2012). Irisin is also correlated with lipid and carbohydrate metabolism (Gagging M et al, 2017). Recent work was conducted to sequence analysis, multiple sequence alignment and phylogenetic tree. We have also predicted the secondary structure and 3D homology molecular model structure of zebrafish irisin protein. The multiple sequence analysis of zebrafish irisin protein in which we found that out of 207 amino acids residues upto 85 positions are consist of conserved amino acids residues in which 'Val' found mostly conserved in whole members of zebrafish irisin protein. Phylogenetic tree shows closeness between Chain. A. Myokine (human) and zebrafish irisin. And we have also determined secondary structure of *Danio rerio* (zebrafish) irisin protein. Secondary structure prediction graph have shown that zebrafish irisin protein consist of one alpha helix, six major and six minor beta sheets. For model building Chain. A. myokine (human) pdb. Id. 4LSD_A was used as the template, which shown highest sequence identity 77% with zebrafish irisin protein. 3D homology molecular model of zebrafish irisin protein have shown all beta sheets.

MULTIPLE UPSTREAM START CODONS (AUG) IN 5' UNTRANSLATED REGION ENHANCE TRANSLATION EFFICIENCY OF *CRY2AC11* WITHOUT HELPER PROTEIN

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The major cause of destruction of agricultural crops is insect pests. Pesticides play significant role in improving agricultural production, but their haphazard use causes harmful effects on the non-target organisms, human health and ultimately pollute the environment. *Bacillus thuringiensis* produce insecticidal crystals protein during sporulation phase and acts as safer alternative to synthetic counterparts. Cry2A acts as bioinsecticides towards agricultural and public health significant order Lepidoptera and Diptera respectively. The gene *cry2Aa* are positioned at third position (Orf3) in operon. It needs accessory proteins for crystal formation and high yield. Genetic manipulation of *cry2Ac11* gene without helper protein was carried out by optimizing Ribosomal binding site and spacer region (RBS-ATG) in translation initiation region (TIR). As modification in RBS-spacer region couples tunes translation initiation rates. Secondary structure of mutants as well as wild type RNA was analyzed by bioinformatics tools. Secondary structure elements, free Gibbs energy in RBS-AUG region of mRNA significantly effect on expression. Mutants were expressed in *B.thuringiensis* acrySTALLIFEROUS strain. RBS-AUG mutagenesis was analyzed by SDS-PAGE. Expression level of *cry2Ac11* mutant (*mut/RBS2*) was enhanced in which triple start codons AUG was introduced in RBS-AUG region. Secondary structure of *mut/RBS2* mRNA revealed that RBS is present in the single stranded region of RBS in moderately stable hairpin loop ($\Delta G = -8.7$ kcal/mol) that facilitates the interaction of RBS to the complementary 16S rRNA sequences and enhances the translational efficiency. The mechanism of multiple starts codons

(AUG) on hyper expression of recombinant protein without chaperone in prokaryotic expression system still need to investigate and correlate with Eukaryotic scanning system. As expression of *cry* toxin gene size in plants can be considered as limiting factor, so this study is going to solve this problem.

IDENTIFICATION OF CILIATE (*PARAMECIUM* SP.) ON MOLECULAR BASIS AND THEIR TOLERANCE TO DIFFERENT HEAVY METALS

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Unicellular ciliate *Paramecium* that can live in a wide range of habitats like fresh, marine and brackish waters and estuaries. *Paramecium* has the ability to absorb heavy metals by binding the metal ions with special kind of metallothioneins proteins present in them. Fresh water samples were collected from Mardana village near Faisalabad. A wide variety of micro-organisms were found in the samples including ciliates, rotifers and algae etc. From these samples different strains of *Paramecium* were isolated and purified. One of the strains SA-1 was identified on the basis of histone H4 gene sequencing as *Paramecium jenningsi*. Minimum inhibitory concentration (MIC) of cadmium, zinc, lead and copper was checked by growing *Paramecium* sp. in different concentrations of these metals. The MIC of cadmium, copper, zinc and lead was 40µM, 120µM, 800µM and 700µM respectively. Optimum conditions for the growth of *Paramecium* sp. at pH 7.5 and temperature 20°C was recorded. Pattern of growth was observed for isolated *Paramecium* sp. with and without metal stress. It showed resistance even at higher concentrations with a maximum growth at day 20 with 35µM cadmium concentration. With copper, zinc and lead maximum growth was observed at 20µM, 100µM and 600µM respectively.

RAPD BASED APPROACH TO DETECT THE HYBRIDIZATION IN MAJOR CARPS HATCHERY STOCKS OF PUNJAB, PAKISTAN

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Current study was planned to check the genetic purity of *Labeo rohita* and *Catla catla* hatchery stocks through random amplified polymorphic DNA (RAPD) based fingerprinting. A total of 60 specimens; thirty of each *Labeo rohita* and *Catla catla* species was collected from Fish seed Hatchery, Bahawalpur of Southern Punjab, Pakistan. A total of 45 decamer primers were used, out of which eight primers screened have three primers exhibited species-specific fragments. The amplicons produced by a primer ranged from as minimum as 4 to a maximum of 9, with an average of 7 bands per primer. An average total of 8 RAPD bands ranging from 0.5 to 2.36 kb were amplified by three selected species-specific primers. The species-specific fragments; OPA-2 983 bp and OPA-7 1050 bp and OPY-10 2200 bp were homozygous for *L. rohita* and *C. catla* species since their presence was confirmed in almost all specimens of their respective species. All of the individuals examined in these species were identified pure species, first generation, and later generation hybrid forms. Based on species-specific fragment patterns, a total of 19 hybrids were detected in *Catla catla* stock while all the analyzed specimens of *Labeo rohita* were found genetically pure. These findings have significant implications for considering the potential conservation value of species purity. As genetically contaminated captive stock is a threat to the purity of wild populations by deliberate releases or escapes not only through competition for resources but also through gene pool contamination if they reproduce in the wild. 8.1 to 13.71 polymorphic bands per primer were amplified. The polymorphic bands in these populations ranged from 56.4 to 59.6%. Polymorphic bands per lane within populations ranged from 4.88 to 5.3%. The similarity within the population from wild varied from 0.40 to 0.83 with a mean \pm SE of 0.57 ± 0.08 . The Jaccard's similarity coefficient ranged from 0 to 0.27. At 0.06 similarity coefficient, two major clusters were formed, which indicates that the genotypes belonging to same clusters were genetically similar and those belonging to different

clusters were dissimilar. Significant ($P < 0.05$) population differentiation indicated some degree of intra- and inters- population genetic variations in two populations of catfish. This might be due to difference in habitat and breeding strategies between the two populations.

SUSCEPTIBILITY OF RS143384 POLYMORPHIC SITE ON GDF5 GENE WITH OSTEOARTHRITIS ONSET

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GDF5 gene regulates the expression of GDF5 protein and played a crucial role in the morphogenesis of tendon, ligament, and bone. Any mutation in this gene correlated to the abnormal development of joints ultimately leading to Osteoarthritis. Therefore the present study was conducted with an aim to identify the polymorphism on GDF5 gene in OA patients of Lahore division. For this purpose a case control study was conducted on 30 patients and 30 controls. Genotypes were identified by sequencing. Allelic and genotypic frequency of rs143383, rs143384 and identified novel SNP was significantly associated with OA in studied population. The polymorphism on rs143383 polymorphic site led to the change of Arginine into tryptophan whereas novel SNP led to the change of Arginine into Serine. Haplotypes CCCT, CTCT, TCCT, TTCA, TTCT were found to be significantly associated with disease onset whereas the haplotype CTCA was found to be protective against disease development. In conclusion GDF5 gene polymorphisms were identified as susceptible regions for OA onset in population of Lahore division.

DNA POLYMORPHISM ANALYSIS OF PIGEON (*COLUMBIA LIVIA*) IN TEHSIL PALANDRI, DISTRICT SUDHNOTI, AZAD JAMMU AND KASHMIR, PAKISTAN

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The study was undertaken to examine the DNA polymorphism in different populations of pigeon (*Columbia livia*) on the basis of plumage colour. Seven random amplified polymorphic DNA (RAPD) markers were used to examine the genetic variability and relationships among ten different populations of pigeon. The sample was collected from various localities of tehsil Palandri, District Sudhnoti, Azad Jammu and Kashmir, Pakistan. High quality of DNA extracted from newly plucked feathers of pigeon (*Columbia livia*) by using slightly modified phenol-chloroform method. The isolated DNA was used for randomly amplified polymorphic DNA (RAPD) analysis. All selected markers showed polymorphic bands and exhibited DNA polymorphism among different population of *Columbia livia*. Seven RAPD markers produced a total of 53 amplified bands, among these, 22 were polymorphic with an average of 44.34 percent polymorphism. From the dendrogram analysis, four clades (I to IV) were clearly observed which indicates that some samples are closely related to each other. Clade I shows the closely resemblance of sample 1, 5 and 7. Clade II shows the resemblance among samples 4, 2 and 9. On the other hand, clade III and Clade IV show the closely association of samples 3 and 8, and 9 and 10. It was observed that Clade III and IV are closely related, while Clade I shows maximum variation with the other clades. It was concluded that similarity occurred due to cross breeding of population, migration of birds, food availability, habitat and climate change are the major factors that involved in the polymorphism of same populations. This study would be helpful to determine the genetic characteristics or features for this agricultural and economically significant species. This will also help to develop and optimize the noninvasive laboratory methodologies and conditions for further studies on different threatened bird's species found in Azad Jammu and Kashmir, Pakistan.

MODERN TAXONOMIC TOOL BY USING OF Cyt-B AND Pol GENE FOR THE EVOLUTIONARY STUDY OF WASP

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Present study describes the phylogenetic reconstruction of wasp species found in the Hazara region of Pakistan. The investigation was based on nucleotide sequence analysis of specific markers: Cyt-B and Pol genes. A total of 2500 samples were carried out which represented 19 species from 3 subfamilies including: Eumeninae: six species, Polistinae: six species and subfamily Vespinae: seven species. Using partial sequences of Cyt-B gene, *D. conoideum*, *D. esuriens* showing the same group 100% of homology, whereas *A. a. bangalensis*, *D. dimidiatipenne* 86% and *A. flavescens*, *R. quinquecinctum* 79%. In Polistinae species, *P. indicus*, *P. wattii* showing 100%, *P. olivaceus*, *P. rothneyi* 90-89%, whereas *P. stigma* 87%. *Vespa mandarina*, *V. orientalis* representing the same group 100% homology, whereas *V. analis*, *V. basalis* 84% and *Vespa tropica* 81% subfamily Vespinae. With the use of partial sequence of Pol gene, species of the subfamily Eumeninae, *A. a. bangalensis*, *R. quinquecinctum* showing 96% homology, whereas *D. conoideum* 35% representing an out group. Members of the subfamily Polistinae, *P. indicus*, *P. wattii*, *P. olivaceus* showing 100% homology, whereas *P. rothneyi*, *P. stigma* 99% and *Ropalidia brevita* 95%, subfamily Vespinae, *V. analis*, *V. orientalis* 100%, whereas *V. mandarina*, *V. tropica* 99%. The result shows nine species: *Delta conoideum* *D. esuriens*, *Anhynchium abdominale bangalense*, *Rhynchum quinquecinctum*, *Antodynours flavescens*, *Ropalidia brevita*, *P. olivaceus*, *P. stigma* and *V. mandarina* were new record for Hazara region whereas four species: *V. mandarina*, *Anhynchium abdominale bangalense*, *Rhynchum quinquecinctum* and *Antodynours flavescens* were new record from Pakistan.

BISPHENOL A TRIGGERS PULMONARY INFLAMMATION LEADING TO MAST CELL INFILTRATION AND TISSUE PROTEIN PROFILING

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Concern is mounting regarding the human health and environmental effects of bisphenol A (BPA), a hormone-disrupting chemical used in plastic and epoxy resin industries. This study was designed to investigate BPA caused alterations in lungs and liver. Study was carried out on male Wistar rats. They were divided into five groups with five rats per group. Group I was named as control group. Group II and III were received BPA (10mg/kg body weight/day) for 6 weeks and 12 weeks respectively. Group IV and V were given BPA (25mg/kg body weight/day) for 6 weeks and 12 weeks respectively. Lungs were taken and processed for histopathology, trace metal content, lung protein profile and liver to investigate expression of hepatic pro inflammatory cytokine (TNF- α). Groups II and III showed disrupted lung architecture with collapsed alveoli, inflammatory cellular infiltration, congested thickened pulmonary vessels, extravasated red blood cells, and collagen fiber deposition. There was a significant increase in number of mast cells of considerable size in inflamed alveoli and bronchiole. These effects were more pronounced in group IV and V. A decrease in the zinc levels and increase in copper and iron levels were found in the treatment groups in comparison with control group. Two types of lung proteins of molecular weight 72 kDa (Type IV collagenase) and 109 kDa (nucleolin) were resolved on the SDS-PAGE. Comparative study of resolved proteins showed altered expression of resolved proteins in all BPA exposed groups in comparison of control group. Gene expression study of TNF- α exhibited highly significant elevation in hepatic TNF- α of all groups i.e. group II (0.51 ± 0.13 folds), group III (0.61 ± 0.05 folds), group IV (1.02 ± 0.08 folds) and group V (1.83 ± 0.03 folds) in contrast of control group when analyzed by one way

ANOVA ($p < 0.0001$). Prolonged administration of BPA caused dose-dependent lung and liver damage so the use of BPA should be prohibited in plastic synthesizing industries and specious handling of plastic containers should be evaded to lessen health hazards.

POTENTIAL OF *BACILLUS SUBTILIS* FOR HETEROLOGOUS EXPRESSION OF HUMAN *INTERLEUKIN-2* (*hIL-2*) GENE

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Interleukin-2 is a cytokine signaling molecule which plays an important role in our immunity. Molecular weight of this cytokine is 15kDa, consists of receptor chains alpha, beta and gamma chains which is important to stimulate its signal mechanism. It proliferates and differentiates of T cells, B cells, natural killer cells and macrophages dependent cytotoxicity. There are considerable research efforts which are ongoing for the development and purification of novel heterologous gene expression system with superior growth and protein purifications. These heterologous proteins were commonly formed in inclusion bodies in *E. coli* which requires tedious purification and refolding. Now it has been analyzed that *Bacillus subtilis* can be the better host for heterologous protein production because it is GRAS status and does not produce proteins in inclusion bodies. The complete mRNA sequence of human *interleukin-2* gene consisted of 471 nucleotides. The gene of human *interleukin-2* was synthesized and used in further steps. Then amplified product was cloned in pTZ57/RT. Confirmation was done from restriction analysis with PstI and SalI. Sub cloning was done with pSTAB-synhIL-2 recombinant vector under the control of cytI promoter and transformed in *E. coli* DH5 α . So in this study we tried to get an expression of *SynhIL-2* in *B. subtilis* KO7 and a strain of BT4Q7 simultaneously. Unfortunately, transformation was not successful in KO7 but *SynhIL-2* protein was expressed and secretes in culture medium BT4Q7. In this present study we evaluated the potential of BT4Q7 to express heterologous proteins.

PRODUCTION OF RECOMBINANT HYBRID CRY1AC PROTEIN AND ANALYSIS OF EFFECT OF RECOMBINATION ON THE TOXICITY OF PARENT PROTEIN

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Bacillus thuringiensis crystal proteins have insecticidal activities against many notorious pests of economically important crops. Cry1Ac is one of the most widely used 3d-Cry proteins. Domain-II and Domain-III recognize different insect gut proteins and determine the target specificity. We constructed a hybrid protein with domain-I and II of Cry1Ac and domain-III of Cry2Ac7 to check the effect of domain swapping on the target range and extent of toxicity of Cry1Ac. Domain I and II encoding DNA nucleotide segments of *mcry1Ac* and domain III of *cry2Ac7* were amplified by the logarithmic procedure (PCR) with the introduction of specific restriction sites and stop codon at the required regions. The plasmid DNA of pET28a(+) expression vector was used to clone the inserts using nuclease restriction enzymes in two steps. *E. coli* BL21 CodonPlus (DE3)-RIL cells were transformed with Cry1.2Ac recombinant plasmid and protein was overexpressed using 1 mM IPTG for 6 hours in LB medium at 37 °C. Protein inclusion bodies were denatured in urea and on-column refolded and purified using Ni-NTA resin. Imidazole eluted purified 68 kDa refolded protein was trypsin digested and purified by anion exchange chromatography. The activated toxin was bio-assayed against *A. gemmatilis* and *C. includens* first instar larvae by diet overlay method. The bioassay plates were placed a 27 °C, 70-80 % humidity and 14:10 h light: dark photoperiod. Mortality was recorded after seven days and LC₅₀ was determined, using Leora software. Cry1.2Ac was found to be highly toxic to *A. gemmatilis* but nontoxic to *C. includens* in contradiction to the parent protein. This result emphasizes the importance of Cry1Ac Domain-III for activity against *C. includens*.

ANTIBODIES AGAINST HEPATITIS C VIRUS 3a ENVELOPE GLYCOPROTEINS E1 AND E2 FOLLOWING CLONING, EXPRESSION AND PROTEIN PURIFICATION IN PAKISTAN

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Hepatitis C is a major global threat as more than 3% of the world population and 10% of the Pakistani population is chronically infected by Hepatitis C Virus (HCV). It may lead to liver cirrhosis, hepatocellular carcinoma (HCC) and eventually death. Current therapy has limited efficacy, more side effects and heavy cost. The present study is focused on the development of recombinant vaccine against HCV by utilizing envelope glycoproteins E1 and E2 of HCV to combat this disease. Blood samples were collected from the HCV 3a infected patients from local area. Total RNA was extracted and cDNA was synthesized through random hexamers. Two sets of primers comprising of outer and inner primer were designed from Los Alamos. Nested polymerase Chain Reaction was carried out for E1 and E2 gene amplification. Sequencing confirmed the amplified genes E1 and E2 as HCV envelope glycoproteins. Cloning was performed by using pPTG19 vector. Both E1 and E2 glycoproteins were expressed using pET21a expression vector in BL21 strain of *E. coli* as expression host. The E1 protein, expressed in soluble form, was purified through salting out procedure followed by anion exchange FPLC. The E2 protein, expressed in insoluble form, was purified by repeated sonications with 20mM Tris-Cl, 5mM NaCl and 0.01% Triton followed by denaturation and refolding through fractional dialysis with Urea. The purified proteins were injected into the rabbits and antibodies were allowed to raise which were confirmed through ELISA in rabbits as well as in human sera. It is concluded from the current study that purified HCV 3a envelope glycoproteins E1 and E2 have significant antigenic activity and can be used as recombinant vaccines against HCV infection.

INCIDENCE OF JAK2 EXON 12 MUTATIONS INCLUDING A NOVEL MUTATION IN V617F-NEGATIVE POLYCYTHEMIA VERA PATIENTS FROM PAKISTAN

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The gain of function mutation V617F in exon 14 of Janus associated kinase 2 (JAK2) gene is frequently detected in patients diagnosed with polycythemia vera (PV). However in many investigations, V617F negative PV patients have been reported to harbor mutations in exon 12 of JAK 2 gene. We investigated 24 patients with PV (diagnosed following 2016 WHO guidelines) to detect V617F mutation through allele specific PCR. The frequency of which was found to be 19/24 (79.2 %). Later on JAK2 exon 12 was amplified by conventional PCR in V617F negative patients and subjected to sequence analysis. A total of 03 mutated sites in exon 12 were detected in only two V617F-negative patients 2/5 (40%). All three substitutions were heterozygous i.e. F537F/I found in both patients and R528R/T, which is a novel mutation. Hematological parameters of individuals harboring mutations do not vary significantly than rest of the PV patients. Previous history and 2.3 years of follow-up studies reveal 15-year survival of this group of patients (n=24) to be 68%. Mean TLC of the study cohort was $17.6 \pm 9.1 \times 10^9/L$, mean platelet count was $552 \pm 253 \times 10^9/L$, mean hemoglobin was 16.9 ± 3 g/dl, mean corpuscular volume (MCV) was 77.2 ± 13.0 fl and mean corpuscular hemoglobin (MCH) was 25.6 ± 3.9

pg. This is the very first attempt from Pakistan to screen JAK2-exon 12 mutations in PV patients. We further aim to investigate Jak2 exon 12 mutations in larger number of PV patients to assess their clinical relevance and role in disease onset, progression and transformation.

TWO SILENT MUTATIONS IN mRNA OF ENDOGLUCANASE (CEL6A) FROM *THERMOBIFIDA FUSCA* ENHANCED ITS EXPRESSION IN *E. COLI*

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The sequence and structure of mRNA plays an important role in solubility and expression of the translated protein. To divulge the role of mRNA secondary structure and its thermodynamics in the expression level of the recombinant endoglucanase in *Escherichia coli*, 5'-end of the mRNA was thermodynamically optimized. Molecular engineering was done by introducing two silent synonymous mutations at positions +5 (UCU with UCC) and +7 (UUC with UUU) of the 5'-end of mRNA to relieve hybridization with ribosomal binding site. Two variants of glycoside hydrolase (GH) family 6 endoglucanase, wild type (*cel6A.wt*) and mutant (*cel6A.mut*) from *Thermobifida fusca* were expressed and characterized in *E. coli* using T7 promoter-based expression vector; pET22b(+). Enhanced expression level of engineered construct (*Cel6A.mut*) with $\Delta G = -2.7 \text{ kcal.mol}^{-1}$ was observed. It showed up to 45% higher expression as compared to the wild type construct (*Cel6A.wt*) having $\Delta G = -7.8 \text{ kcal.mol}^{-1}$ and 25% expression to the total cell proteins. Heterologous protein was purified by heating the recombinant *Escherichia coli* BL21 (DE3) CodonPlus at 65°C. The optimum pH for enzyme activity was 6 and optimum temperature was 60°C. Enzyme kinetic parameters K_m and V_{max} were found to be 56 mg ml⁻¹ and 25 U min⁻¹ respectively. It is suggested from this study that mRNA secondary structure engineering can be a good tool for improved expression and enhanced production yield of proteins.

THREE LOCI MOLECULAR CHARACTERIZATION OF LOCALLY ISOLATED *PARAMECIUM* SPECIES

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Ciliated protozoan are an important bioindicators for pollution and have long been used for environmental biomonitoring, particularly in water purification plants and in activated sludge processes. Among ciliates *Paramecium* has become a privileged model for the study of "species problem" particularly in the case of "*Paramecium aurelia* complex" that has been intensely investigated. Despite extensive studies taxonomy of *Paramecium* is still changing. For this purpose, fragments of 18S rDNA, ITS1-5.8S-ITS2-5'LSU rDNA, COII, Hsp70 and Histone H4 genes were used as molecular markers for the phylogenetic analysis of ten locally isolated strains of *Paramecium* species including a standing-alone FT8 strain previously isolated by Shakoori *et al.* (2014). The nucleotide sequences of PCR products of different molecular markers of various isolates (FT2.1, FT3.1, FT4.1, FT5.1, FT6.1, FT7.1, FT9.1, FT10.1 and FT11.1) were compared with the available sequences of these markers in other *Paramecium* species from GenBank. Phylogenetic trees based on all molecular markers showed that all nine strains (FT2.1, FT3.1, FT4.1, FT5.1, FT6.1, FT7.1, FT9.1, FT10.1 and FT11.1) had very close relationship with *P. primaurelia* except for FT8 strain. FT8 showed its unique position in comparison to all other species in the phylogenetic trees, so became the main focus of our study. Phylogeny of this strain was further carried out with Piggymac sequence, where it again behaved differently as compared to other species. Sexual behavior, immaturity and maturity periods of FT8 were analyzed by performing daily reisolations that revealed three unique characteristics of this strain; 1) autogamy as only source of the exchange of genetic material 2) no clumps formation before conjugation that is the prerequisite of sexual process, 3) selfing among reactive cells. Second and third characteristics of this species turned our attention towards its mating types

("odd" 0 and "even" E). Why agglutination does not occur in this strain like all other *Paramecium* species? Or maybe there are circadian rhythms of mating types? In order to resolve this mystery, an experiment following circadian rhythms of completely light and completely dark cycles was performed multiple times during a period of one month. However, every time no conjugation upon mixing but selfing in the original cultures was observed. Results of this study could not prove the existence of mating types in FT8 strain but off course detailed analyses at genetic level is required for precise knowledge.

6. PHYSIOLOGY

RELATIONSHIP OF KIDNEY FUNCTION WITH OBESITY IN OUR LOCAL POPULATION

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The kidneys play role in the body, providing an excretory mechanism as part of the urinary tract to eliminate waste of metabolism and so on. Obesity is known to increase the risk of a number of chronic diseases and is the most common nutritional disorder. Chronic obesity also causes marked structural changes in the kidneys that eventually lead to a loss of nephron function. In this cross-sectional study, the total 75 subjects were enrolled that were divided into two groups, Obese (n = 50) and Control (n = 25). Kidney function was estimated using serum creatinine, blood urea and eGFR was calculated by MDRD equation. The mean serum creatinine of obese individuals was 2.35 ± 0.233 (mg/dl) and that of control was 0.92 ± 0.25 (mg/dl) with significant difference between the groups. The mean blood urea of obese individual was 65.5 ± 4.89 (mg/dl) and of control was 32.6 ± 1.26 (mg/dl) indicating significant difference. The mean MDRD estimated by GFR of obese subjects was 53.62 ± 4.45 and that of control was 82.90 ± 4.31 , with significant difference between the groups. It was concluded that with the rise of BMI the kidney function decreases as revealed by low eGFR in obese group.

POTENTIAL ANALGESIC, HEMOLYTIC AND HISTOPATHOLOGICAL EFFECTS OF N-ALKYL-3-METHYLPYRIDINIUM BROMIDES

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Several formulations of analgesics are although regularly used all world over, associated side effects bias the selection of a potent drug for an effective prescription. It has imperative therefore to formulate and design the most effective, non-addictive and least toxic drug for pain relief. As pyridine ring in one of the most of the naturally occurring in various compounds, pyridine derivatives are considered potential candidates for pain relieving effect in addition to previously reported properties like anti-inflammation, anti-cancer and anti-microbial. In the current study, N-alkyl pyridinium bromides were evaluated for their analgesic activity using the hot plate, tail flip and tail clip tests. Healthy male BALB/c mice (n=135) were divided into different treatment and control groups (n=15/group). All synthetic compounds (SF6, SF8, SF10, SF12, and S14) were administered intramuscularly (i.m) to five treatment groups respectively at the dose of 10 mg.kg^{-1} b.w. As SF8 was highly toxic at 10 mg.kg^{-1} dose, a low dose of 2.5 mg.kg^{-1} b.w was also tested. Positive control mice were treated with equivalent doses of ketobrufen. Histopathology was studied for liver and kidney tissues. Results were compared statistically at $P < 0.05$. Additionally, hemolytic activity of these compounds was separately performed on blood samples collected from human volunteers (n=5) with O +VE blood group. Blood was drawn in heparin vacutainers. RBCs were separated and treated with different molar concentrations (75, 100, 125, 350, 450, 650 μM in ethanol) of each compound. Hemoglobin content was measured spectrophotometrically at 540 nm. Dose-dependent inhibition or IC₅₀ was also determined for each compound. All the compounds (SF6, SF10, SF12 and SF14) showed significantly greater mean latency response as compared to the ketobrufen treated positive control and distilled water treated control ($p < 0.001$). For the hot plate test, SF6 and SF10 treated animals ($p < 0.001$) showed maximum latency response. Similarly, in tail clip test, significantly increased mean latency responses were observed for all compounds as compared to Ketobrufen and distilled water treatment. Among these, SF14 and SF12 ($p = 0.004$) showed maximum latency response indicating its greater analgesic effects. Likewise, for tail flip test, treatment groups demonstrated their analgesic effects in comparison to both positive and negative controls with SF10 and SF12 showing maximum ($p = 0.011$) latency response. Furthermore, analgesic activity of SF8 treatment in tail clip and tail flip tests at low dose (2.5 mg/kg) also showed significant effect against both positive and negative control groups. Whereas, for hot plate test, SF8

showed significant analgesic effect only against negative control ($p < 0.001$). Histologically, except SF8, other compounds appear to have caused least toxic effects in liver and kidney tissues. All the compounds showed dose dependent increase in hemolytic activity for human blood. Based on IC₅₀ values, compound SF12 exhibited maximum hemolytic activity and was ranked 1 in the list. SF6 exhibited the lowest IC₅₀ value and was ranked at 5. The hemolytic activity of all the compounds was in the following order SF12 > SF8 > SF14 > SF10 > SF6. The current study concludes that N-Alkyl-3-methylpyridinium bromides can provide more effective and less toxic alternatives of analgesic drugs available in the market.

COMBINED EFFECT OF EPLERENONE AND COCOA POWDER IN IMPROVING BLOOD GLUCOSE AND ELECTROLYTE EXCRETION BY THE KIDNEYS OF ALLOXAN INDUCED DIABETIC ALBINO MICE

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The present study was aimed to investigate the effect of eplerenone, a selective aldosterone blocker and cocoa powder, a natural antioxidant and antihypertensive on kidneys of alloxan induced diabetic albino mice. Control group (n=8) orally received saline (0.9% NaCl) daily. Experimental group (n=24) received alloxan (150 mg/Kg body weight) for three days for the induction of diabetes. After the induction of diabetes, experimental group was then divided into two subgroups i.e., (i) Diabetic control and (ii) diabetic treated. Diabetic control group (n=8) received saline (0.9% NaCl). The eplerenone treated group (n=8) received eplerenone (8.0 mg/kg body weight) daily and eplerenone+ cocoa powder treated group (n=8) received eplerenone daily and cocoa powder (1g/kg body weight) on every third day for four weeks. Blood samples of 4 animals from each group were collected after every two weeks, and plasma was separated. Sodium and potassium excretion checked from plasma. Data was analyzed statistically. The result showed that eplerenone lowers the blood glucose level and diminishes the diabetic complications after four weeks of treatment. The therapeutic and remedial effects of eplerenone were improved when used in combination with cocoa powder and act more effectively against blood glucose level after four weeks treatment with highly significant results ($p < 0.01$). Hence it is concluded that drug and herb used in combination is more potent.

PLASMA PROTEINS AND COMPONENTS IN WORKERS OCCUPATIONALLY EXPOSED TO HAZARDOUS CHEMICALS AT AUTOMOBILE WORKSHOPS AND PETROL FILLING STATIONS

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Automobile mechanics and petrol station attendants are continuously exposed to many hazardous chemicals, of which, benzene and lead are top listed. The present study was designed to evaluate the serum protein alterations in automobile mechanics and petrol station attendants in comparison to control subjects. The present study is based on 29 samples of automobile workers, 19 samples of petrol pump attendants and 24 samples of control subjects. Serum protein analysis was carried out to analyze the effect of inhaling vapors or fumes of different hydrocarbons emitted at petrol pump stations and automobile workshops on total protein (TP), Albumin (HSA) Total globulins, gamma globulins and non-gamma globulins level of controls and test subjects, by use of Clinical Chemistry Analyzer 5010 in Physiology Laboratory, Department of Zoology, Punjab University, Quaid-e-Azam Campus, in Lahore. Statistical presentation of the data obtained from each parameter of the study has been done. One-way analysis of variance (ANOVA) Tukey's Post hoc Multiple Comparison Analysis was employed for the comparison of variations among comparable groups. There were significant variations in parameters of study amongst three groups due to the exposure of workers to hydrocarbons and gasoline vapors at work place. Total proteins increase significantly compared to control group in petrol pump attendants whereas highly significant decrease was noticed in albumin. Gamma globulins decrease significantly in both groups compared to control group. Highly significant decrease in gamma

globulins parallel with increase in non-gamma globulins may be regarded as a indication of immunotoxicity

**HORMONAL MASCULINIZATION IN COMMON CARP (*CYPRINUS CARPIO*)
BY IMMERSION TECHNIQUE**

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The trial was conducted with the aim to masculinize common carp (*Cyprinus carpio*) by egg immersion technique. Specifically, to evaluate the effect of different hormone concentrations (17 α -methyltestosterone @ HC: 150, 300, 450 and 600 $\mu\text{g l}^{-1}$), immersion times (IT: 24, 48 and 72 hrs) and their interaction effect (HC x IT) on the hatching percentage of *Cyprinus carpio* eggs, percent survival and percent of males. Results showed that hatching percentage decreased with increased IT likewise, survival of treated fry was affected by increasing the IT ($P < 0.001$). The main interaction effect of (HC x IT) showed that the highest percent of male individuals 95% was attained at 450-600 $\mu\text{g l}^{-1}$ HC at 72 hrs IT, followed by 88-92.50% at 150-300 $\mu\text{g l}^{-1}$ HC at 72-hrs IT, 87.50% at 48-hrs IT for all hormone treatments, and lowest 47.50% was recorded in control ($P < 0.05$). Increased percent male of *Cyprinus carpio* was obtained with increasing HC across all IT. It was observed that the immersion treatment at 600 $\mu\text{g l}^{-1}$ for 72 hours is more effective to change the sex ratio of pre hatch *Cyprinus carpio*. From this experimental trial it was concluded that sex inversion of *Cyprinus carpio* by egg immersion is an alternative technique of masculinization by oral administration of hormone in feed.

**EFFECT OF SEASONAL VARIATION ON GONAD MORPHOLOGY OF
CLUPISOMA NAZIRI IN RIVER INDUS.**

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The effect of different seasons on histomorphological structure of gonads in *Clupisoma naziri* was studied. A total of 43 mature fish of both sexes were sampled from the river Indus and its tributaries in Khyber Pakhtunkhwa and northern Punjab, Pakistan. Spring season was the preparatory phase for both testis and ovaries. Thick tunica albuginea and rapid spermatogenesis in testes while appearance of cortical alveoli or yolk vesicle in cortex of cytoplasm in ovaries was observed. The gonads were observed fully matured in summer season showing the testicular lobules filled with spermatozoa and some spermatogenic cells; ovaries were filled with mature ovarian follicles. Free oozing of spermatozoa in testes and ovaries packed with fully grown eggs are the distinct features of spawning phase. Therefore summer was considered as spawning season of *Clupisoma naziri*. The results of gonadosomatic index (GSI) were similar with the histological results of gonads as their values reach to maximum during summer (spawning phase) and showed the lowest value during spring (preparatory phase). Present study revealed that *Clupisoma naziri* breed once in a year during summer season and this information will be helpful in culturing of this economically important catfish in Pakistan.

**RENOPROTECTIVE EFFECT OF INHIBITING RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) BY
CAPTOPRIL VERSUS LOSARTAN ON DRUG-INDUCED ACUTE KIDNEY INJURY (AKI).**

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The present study was aimed to investigate the renoprotective effect of inhibiting renin-angiotensin-aldosterone system (RAAS) by captopril versus losartan on drug induced acute kidney injury (AKI). In this study sixty (60) male albino rats divided into six groups (10 rats/group); control, AKI, AKI+captopril, AKI+losartan, captopril and losartan have been used. GFR (glomerular filtration rate) and SBP (systolic blood pressure) have been measured. For measurement of MCP-1 (monocyte chemoattractant protein-1), urea, NGAL

(Neutrophil gelatinase –associated lipocalin, ICAM-1 (intercellular adhesion molecule -1), cystatin-C and creatinine serum samples were collected. Kidney tissues for measurement of tissue of KIM-1 (Kidney injury molecule-1), MDA (malondialdehyde) and renal expression of megalin were removed. Both losartan and captopril attenuated drug induced acute kidney injury (AKI) as revealed from the measured tissue parameters and serum. As compared to losartan, captopril was found to be superior on normalizing KIM-1, megalin and ICAM-1 while as compared to captopril, losartan improved more GFR. In AKI, groups treated with captopril and groups treated with losartan showed no significant difference in such type of measured parameters. Both losartan and captopril attenuated drug induced AKI (Acute Kidney Injury) as concerned from the tissue parameters and measured serum. Captopril was found to be superior to losartan on normalizing megalin, KIM-1 and ICAM-1, however, losartan has improved GFR (glomerular filtration rate) more as compared to that of captopril, while other parameters in AKI (Acute Kidney Injury) groups which were treated with losartan and groups which were treated with captopril showed no significant difference. For histopathological evaluation of the renal tissue for further examination of the state of medulla and the cortex of the kidney we recommend more studies by other blockers of RAAS (Renin Angiotensin Aldosterone System).

EVALUATION OF CIRCULATING BIOCHEMICAL AND OXIDATIVE STRESS MARKERS IN PATIENTS WITH THYROID DYSFUNCTION UNDER INTERFERON THERAPY

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Hepatitis C is a most common disease affecting large population of the world. Infected patients under Interferon therapy grow thyroid dysfunction. Normal Growth, Development, Function, Metabolism of Cells & organs is regulated by Thyroid hormones i-e Thyroxine, Triiodothyronine. Liver, Heart have more damage than other organs and Thyroid Hormone modify respiratory chain components of mitochondria which may increase production of Reactive O₂ causes oxidative stress. The present study is conducted on 70 patients of Hepatitis C under Interferon Therapy and 60 controls. Venous blood is collected from each participant and analyzed for thyroid profile (T₃, T₄ and TSH), Oxidative Stress markers (MDA, GSH, CATALASE and SOD), Liver Function Tests(Bilirubin, ALP, ALT and AST), Cardiac Enzymes (CPK, CKMB and LDH), Renal Profile (Urea and Creatinine), lipid profile (total cholesterol-TC, triglyceride-TG, high density lipoprotein – HDL, Low density lipoprotein-LDL) and Electrolytes(Calcium- Ca²⁺, Magnesium- Mg²⁺, Phosphorous- Po₄³⁻, Sodium- Na⁺, Potassium-K⁺) are estimated. **Results:** Statistical analyses are performed by using SPSS for Windows version 20.0. Mean values of (T₃, T₄) and (GSH, CATALASE and SOD) are significantly lower in patients with Thyroid Dysfunction under INF therapy (P<0.000) as compared to controls. Mean values of Serum (TSH), (MDA), Liver Function Tests(Bilirubin, ALP, ALT and AST), Cardiac Enzymes (CPK, CKMB and LDH), Renal Profile (Urea and Creatinine), lipid profile (total cholesterol-TC, triglyceride-TG, high density lipoprotein-HDL, Low density lipoprotein-LDL) and Electrolytes(Calcium- Ca²⁺, Magnesium- Mg²⁺, Phosphorous- Po₄³⁻, Sodium- Na⁺, Potassium-K⁺) are also higher in patients (P<0.000) and also statistically significant. Some variables are positively correlated with T₃ and T₄ values. **Conclusion:** HCV patients with Thyroid Dysfunction is associated with raised oxidative stress and circulatory biochemical markers.

DETERMINATION OF PHYSIOLOGICAL, BIOCHEMICAL AND ANTI-OXIDATIVE STATUS IN PATIENT SUFFERING FROM TYPE -1 DIABETES MELLITUS

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Type 1 diabetes mellitus disorganization of glucose equilibrium distinguishes by autoimmune disruption of the insulin producing pancreatic β -cell that constantly leads to insulin scarcity and resulting hyperglycemia.

The symptom consists of excessive appetite, excessive thirst, and loss of weight, debility, muscle convulsion, constipation, obscured vision, candidiasis and polyuria. Individuals with prolonged existing type 1 diabetes are affected to micro vascular entanglements and macro vascular complication for instance. 60 diabetic patients and 50 Samples of healthy individuals were taken from Nawaz Sharif Hospital. 5.0 ml blood sample was taken. At 4000 rpm for 10 minutes the centrifugation of blood will be done and serum was separated. Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), Malondialdehyde (MDA), Nitric oxide (NO), micronutrients (Vitamin A, Vitamin C and Vitamin E) and Electrolytes was determined. MDA level is progressively higher in Diabetes type 1 patients (14.01 ± 0.06) as compared to control persons (1.27 ± 0.21) (P- Value 0.000). GSH status is note ably reduced in diabetic patients (0.15 ± 0.05) as compared to normal (6.24 ± 0.33). (P- Value 0.000) Comparable anti-oxidant catalase is reduced (2.82 ± 0.04) in affected individuals as comparison to normal individuals 4.19 ± 1.09 (P-Value 0.000). SOD level is remarkably marked up to (13.52 ± 3.21) in susceptible persons as compared to normal (2.15 ± 0.23) (P-Value 0.000). Vitamin A level is markedly reduced to (1.62 ± 0.26) in patients as compared to healthy individuals (7.18 ± 0.33) (P-Value 0.000). Vitamin C value is also marked down in patient's 0.45 ± 0.07 in comparison to normal individuals 6.23 ± 1.08 . (P-value 0.000). Type 1 diabetes patients particularly reduced amounts and competency of antioxidant protections due to elevated consumption of specific anti-oxidant components such as low level of intracellular glutathione and Catalase. And primarily low levels of vitamin A, vitamin E and vitamin C and exalted level of MDA, SOD and NO.

**INTERRELATION BETWEEN PHYSIOLOGICAL, BIOCHEMICAL AND
NTI-OXIDATIVE BIOMARKER IN SCHIZOPHRENIC PATIENTS RECEIVING
ANTI-PSYCHOTIC DRUGS FROM LAHORE, PAKISTAN**

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Schizophrenia is a persistent, serious and ruining mental ailment that influences individuals over the record. It alters thinking, action and emotion of person. It is the mental disorder characterized by array of symptoms including variation in perception, delusion, hallucination, disorganized thought and speech and impaired cognitive ability. Oxidative stress is elucidated by elevated free radical production and diminished capacity of antioxidant system. Oxidative stress theory is securing attention in schizophrenia and it has detrimental effect on socio and neurocognitive abilities in schizophrenia. Sixty patients of schizophrenia and fifty clinically healthy individuals of matched age group were acceptable for inclusion in the study. 5.0ml blood sample were taken and subjected to centrifuge for serum isolation at 3000-4000rpm for 10-15mins. The level of MDA, CAT, SOD, GSH, micronutrients (Vitamin A, C, E) and electrolytes Na⁺ and K⁺ was estimated. MDA level in schizophrenic patients was elevated remarkably (12.02 ± 0.14) as compare to healthy person (1.27 ± 0.28) and statistically significant (p-value <0.05). The level of GSH in schizophrenic patients was declined (0.14 ± 0.06) as compare to control healthy individual (6.38 ± 0.19) and statistically significant (p-value <0.05). CAT level fallen off in SZ patients (2.81 ± 0.03) as compare to healthy individual (4.9 ± 1.03) and statistically significant (p-value < 0.05). The level of SOD was decreased in SZ patients (0.92 ± 0.11) as compare to healthy individuals (2.16 ± 0.33) and statistically significant (p-value <0.05). Our study manifests that SZ patients treated with antipsychotic drug have extremely elevated lipid peroxidation rate that led to high level of MDA and the disequilibrium in oxidants and antioxidant protective system is predominant factor in progression of schizophrenia. Our finding would give new dimension to treatment in addition to available antipsychotic treatment.

FREQUENCY OF ADMISSIONS OF UNCONTROLS HYPERTENSION IN PATIENTS VISITING BACHA KHAN MEDICAL COMPLEX, SHAHMANSOOR, SWABI

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Hypertension is related to heart and blood vessels which cause arthrosclerosis and heart failure and high occurrence of death. Worldwide 92million people are affected. Hypertension is frequently asymptomatic has no warning signs and the affected people do not feel sick. In Pakistan 18% of people is suffer in which 12.5% is effectively controlled. The purpose of this research is to identify the disease its damages and its frequency among the attendance in patient's visiting Bacha Khan Medical Complex Shahmansoor Swabi. A cross sectional survey was conducted from the month of October 2016 to May 2017. The whole study consists of total 162 patients visiting Bacha Khan Medical Complex Shahmansoor Swabi in which females were 110 and males were 52. Age of patients was above than 40 years. When a Gender wise patient is distributed it show high frequency in females that were 110 than males that were 52. In females hypertensive damages is mostly seen due to their obesity. Also divided the number of patients into age wise groups in which high prevalence found in the age of 61 to 70 years patients that were 43 patients and on the basis of month wise distribution high prevalence found in February that were 37 patients. Uncontrolled hypertension can be fatal it can be controlled by few lifestyle changes like Maintain a healthy weight, Strive for a body mass index (BMI) between 18.5 and 24.9, Eat healthier, Eat lots of fruit, veggies and low-fat dairy, and less saturated and total fat, Reduce sodium, Stay under 1,500 mg a day, which is associated with the greatest reduction in blood pressure, Become active, Shoot for 40 minutes of moderate to strong physical activity 3-4 times per week, Limit alcohol. These lifestyle modifications were helpful in reducing hypertension.

ENHANCEMENT OF EXTENDER EXCELLENCE OF FROZEN BULL SEMEN USING A-TOCOPHEROL AS AN ANTIOXIDANT

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Affluent fertility is one of the most imperative and preferred segment of a modern cattle breeding program. Adequate sperm functionality (normal morphology, motility and ability to undergo the events of capacitation) is perquisite for triumphant contact of spermatozoa with oocytes in the female reproductive tract for successful fertilization. During semen preservation process, polyunsaturated fatty acids of sperm membrane are highly susceptible to lipid peroxidation owing to ROS, which damages cell membrane and nucleic acids and eventually decrease sperm function. α -tocopherol has been associated with enhancement of spermatozoa functional excellence that includes motility and viability of spermatozoa by preventing oxidative damage to the plasma membranes. To our knowledge, no precise reports are available on the use of α -tocopherol as an antioxidant in frozen bull semen preservation in the subtropical environment. Accordingly the current endeavor has been elucidated to establish the most positive level of α -tocopherol for cryopreservation of Achai and Holstein Fresian bull semen under the subtropical condition of Peshawar region. Experiments were carried out semen of six matured bulls, three Achai- the indigenous breed and three Holstein Fresian- exotic breed at Government Cattle Breeding and Dairy Farm Harichand Charsadda. Artificial vagina maintained at 42°C was used for collection of Semen from the experimental bulls of either breed and processed independently breed wise. Two successive ejaculates were collected from each bull at weekly interval for 3 weeks in April- May 2014. Gross and microscopic examination of the ejaculates was appraised soon after collection. Semen specimens with above 70% motility were chosen for advance analysis. The semen was split into four aliquots in

which tris-citric acid extender (TCA) supplemented with various concentrations of α -tocopherol was used to reach 0 mgml⁻¹ (group I, control), 0.5 mgml⁻¹ (group II), 1.0mgml⁻¹ (group III), 1.5mgml⁻¹ (group IV), 2.0mgml⁻¹ (group V), 2.50mgml⁻¹ (group VI) and 3.0mgml⁻¹ (group VII). Diluted semen was cooled to 4°C for 2 h, equilibrated for 4 h at 4°C, filled in straws(0.5ml) at 4°C, kept in liquid nitrogen vapors for 10 min and subsequently held in the liquid nitrogen(-196 °C) for assessment. Post-thawed motility of frozen semen straws was assessed using standard procedure. Hot-water bath was used for 30 seconds for Thawing of frozen semen straws at 37°C. Sperm viability and acrosomal integrity were determined by dual staining procedure i.e Trypan-blue and giemsa stains. live and dead spermatozoa were differentiated via the supravital stain trypan-blue whereas giemsa integrity of the acrosomal membrane were assessed through giemsa stain. Similarly the hypo- osmotic swelling (HOS) test was used to assess plasma membrane integrity. The data was statistically analyzed with two-way analysis of variance using SPSS version 16, Chicago, IL, USA. Duncan's Multiple Range Test was applied among the treated groups to make clear the level of significance. During current study, the progressive motility and viability of spermatozoa was significantly higher in 1.5mg or 1.0mg α -tocopherol supplemented groups in comparison with 0.5mg or 0.00 mM. Also current finding indicated that the number of HOST+ve spermatozoa at 1.5 or 1.0 mg was greater in comparison with lower concentration of 0.5 or 0.00mg α -tocopherol. Furthermore, significant difference for acrosomal integrity was recorded in semen samples treated with 1.5 mg α -tocopherol followed by 1mg, 0.5mg and control. The current study demonstrated that 1.0 or 1.5mg ml⁻¹ was the most favorable concentration of α -tocopherol to be added to the TCA extender for improving the quality of frozen-thawed Achai and Holstein Fresian bull semen.

ANTICANCER AND FOLIC ACID ATTACHMENT ON TO CDS QUANTUM DOTS NANOCARRIERS FOR DIAGNOSTICS AND DRUG DELIVERY

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Nanotechnology has revolutionized an emerging field of nanocarriers that has promised in the preparation of cancer therapeutics. The semiconductor Quantum Dot (QDs) composites were used as carrier for targeting drug delivery. Cadmium sulfide (CdS) nanoparticles were synthesized by wet method under hydrothermal conditions and were bridged through sulfur of cysteine residue. Due to high expression of folate receptors on cancer cells, CdS-Cys-NPs were further coated with folate and Dacarbazine (anti cancer drug) by glutaraldehyde and carbodiimide method respectively for targeting folate receptors. The binding of CdS nanoparticles were characterized by FTIR and fluorescent imaging. The binding efficiency of CdS composite and anticancer drug (Dacarbazine) delivery was checked on HeLa cells and characterized by MTT assay and cell apoptosis assays. In histochemical and immunohistochemical studies, fluorescence indicated the binding of CdS composites on HeLa cells. Immunohistochemical studies were also conducted in mouse tumors. Comparative MTT assay further indicated that activity of Dacarbazine is enhanced when used in combination with Cadmium sulfide.

EFFECT OF EXOGENOUS OXYTOCIN ON PREVALENCE AND NATURE OF MASTITIS IN DAIRY BUFFALOES

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To evaluate the effect of exogenously administered oxytocin on prevalence of mastitis and its comparison with other methods of milk let down, a case-control study was designed in which 100 buffaloes

were examined for mastitis. Overall and quarter wise prevalence of mastitis were 26 and 8.08%, respectively. Prevalence was highest (31.43%) in buffaloes having let down of milk with exogenous oxytocin than having let down of milk with calf suckling (24%) and concentrate (20%). Buffaloes housed on brick floor have higher prevalence of mastitis (27.11%) than buffaloes on ground floor (24.39%). Buffaloes have highest mastitis prevalence (33.33%) in their first month of lactation. Mastitis prevalence increased with increasing number of lactations upto 3rd lactation and then declined progressively in succeeding lactations. Buffaloes milked with exogenously administered oxytocin have higher abortion rates (25.71%) than having milk let down with calf suckling (6.00%) and concentrate (0.00%). Microorganisms isolated from quarter foremilk samples of buffaloes with milk let down stimulus with exogenous oxytocin and without oxytocin were *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, coagulase negative *Staphylococci*, *Escherichia coli*, *Corynebacterial* species and *Bacillus* species having 40, 20, 16, 8, 8, 4 and 4% prevalence, respectively. Microorganisms isolated from non mastitic animals were *Streptococcus agalactiae*, *Escherichia coli* and *Bacillus* spp. having 25, 37.5 and 37.5% prevalence, respectively. It was concluded that an association exists between exogenous oxytocin administration and occurrence of mastitis. Thus, an effective control and management programme should be adopted to minimize the prevalence of mastitis in dairy buffaloes.

PREVALENCE OF TYPHOID FEVER IN DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Typhoid fever is a systemic infection which is caused by a bacterium called *Salmonella Typhi*. Typhoid fever is among one of the important public health problem in developing and underdeveloped countries. It is estimated that typhoid fever is the sixth most common cause of death in Pakistan and prevalence of this disease is 412 cases per 100,000 populations per year in Pakistan. The present study was conducted in DHQ hospital Swabi from April to July 2016 to study prevalence of typhoid in Swabi. The results of our study suggest that out of 1020 suspected patients 159 were positive to widal test having percentage of 15.58%. Out of 1020 suspected patient 340 were females and 680 were males. Out of 1020 suspected patients 43 females and 116 males were positive to Widal test with percentage of 12.64% and 17.05% respectively. Our results also suggest that the prevalence of typhoid fever were more in patients having age range from 11 to 20 years followed by people having age range between 21 to 30 years. The prevalence of typhoid is very less in old people have age range between 61 to 70 years. The results of our study revealed that prevalence of typhoid with respect to age range is 21-30 years > 11-20 years > 1-10 years > 31-40 years > 41-50 years > 51-60 years > 61-70 years having percentage 28.65%, 22.58%, 20.27%, 12.14%, 9.72%, 6.52% and 4.03% respectively. The results of our study suggest that typhoid fever is still one of the major health risks in District Swabi. Therefore it is the responsibility of health department and local government to spread awareness in District Swabi about typhoid fever and also improve sanitation system in Swabi. It is also responsibility of provincial government as well as federal government to provide vaccines against typhoid to every DHQ hospital in order to cope against the typhoid fever which is still one of the major health risks in entire Pakistan.

A COMPARATIVE STUDY OF HEMATOLOGICAL VARIATIONS IN HIGH ALTITUDE AND PUNJAB PLAIN RESIDENTS.

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At 3658m, the barometric pressure falls and there are 40% fewer oxygen molecules per breath, thus lowering oxygen supply to the body tissues leading to hypoxia. Residents at high altitude face low partial pressure of oxygen so their hematological profile is different than that of the sea level residents. This study determines the hematological variations in high altitude residents with worsening hypoxemia and their

comparison with Punjab plain inhabitants. Blood samples of 30 healthy high altitude residents were selected from Rawalakot (5300ft), AJK and 30 healthy Punjab plain residents from Lahore (712ft). Hematological profile including WBC count, RBC count, HGB, HCT, PLT count, MCH, MCHC, of the plains and high altitude residents were determined using Sysmex KX-21 hematology analyzer. Inter group comparison was made to access the variations. HGB, DBP, HCT, RBC count, MCH and MCHC showed significant increase in high altitude residents when compared with plains inhabitants. There was non significant increase of WBC count and non significant decrease of PLT count in high altitude residents as compared to the Punjab plain inhabitants. It may be concluded that human body gets acclimatized by adapting to some of the physiological responses; one of them is variable hematological profile, to face the hypobaric hypoxia.

PREVALENCE OF BETA-THALASSAEMIA MAJOR IN AZAD JAMMU AND KASHMIR, PAKISTAN

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Thalassemias are a characterized by several complications due to heritable gene mutations which cause severe anemias and require blood transfusions throughout life. β -thalassemia has been identified as a most prioritized problem among the other haemoglobinopathies, especially, in developing countries. Around 68,000 children are born with different types of thalassemia every year. The purpose of the study was to assess the prevalence of thalassemia in the region of Azad Jammu and Kashmir, Pakistan. The samples were collected from Sheikh Khalifa bin Zayed Al Nahyan Hospital, Muzaffarabad and Combined Military Hospital, Rawlakot. Complete blood picture (CBP), iron, ferritin, unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC), percent saturation of transferrin Erythrocyte porphyrin tests and Hb-Electrophoresis, were performed to exclude hemoglobin disorders other than Thalassemia. Among all types of populations total 281 individuals (38 males and 32 females) were surveyed, 211 (75.1%) were β -thalassemia minor and 70 (24.9%) were found β -Thalassemia major. Among these patients 20 (28.6%) individuals were found to be affected with Hepatitis B or C. It was found that the illness was common in both the genders. High prevalence (75.1%) of Beta-thalassemia in heterozygous state occurred. Individuals (24.9%) were found to be Beta-thalassemia major. Patients with Beta-thalassemia major were also found to be affected with Hepatitis B, Hepatitis C or both in some cases.

ANTHROPOMETRY, BLOOD PRESSURE AND RAMADAN FASTING

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Fasting is an Islamic practice, which caused various metabolic changes in fasting individuals. The aim of present study was to examine the effect of Islamic fasting on different anthropological and physiological changes like body mass index (BMI), Waist hip ratio (WHR), arm and wrist circumferences as well as blood pressure in fasting obese, over weight and normal-weight individuals of both the sexes. This is a prospective study in which 55 fasting Muslim healthy adult males and 55 females in the age group 20-40 years were included. Detailed history, anthropometric measurements and blood pressure was recorded, twice: at first day and on last day of Ramadan. Data were analyzed by one-way ANOVA. Ramadan fasting resulted in a significant decrease in BMI (Kg/m^2) ($P < 0.05$; $P < 0.05$), WHR ($P < 0.05$; $P < 0.05$), SBP (mmHg) ($P < 0.05$; $P < 0.05$), and DBP (mmHg) ($P < 0.05$; $P < 0.05$) of overweight and obese males. BMI and WHR of post-obese females were considerably reduced ($P < 0.05$; $P < 0.05$) as well. Mean values of SBP of Post Ramadan control and

obese were significantly reduced ($P < 0.01$; $P < 0.01$). Post Ramadan control and obese females showed reduced DBP as compared with their pre groups ($P < 0.05$; $P < 0.05$). Ramadan fasting is beneficial in a way that it caused significant reduction in body weight, waist circumference and blood pressure in obese and overweight subjects.

THE ROLE OF INFLAMMATORY MARKERS FOLLOWING RAMADAN FASTING

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Ramadan is an Islamic month during which Muslims abstain from eating, drinking and smoking from dawn to sunset. The aim of present study was to investigate the effect of fasting during Ramadan on plasma adiponectin and TNF- α levels. This prospective observational study was conducted in June-July 2014. A total of 55 females and 55 males whose ages ranged between 20 and 40 years who were fasting during Ramadan were enrolled in the study. Subjects were divided into control, overweight and obese males and females. Anthropometric measurements were taken as well as Fasting venous blood samples were taken at first day of Ramadan and during the last week of Ramadan. Plasma adiponectin and TNF-alpha levels were measured through commercially available ELISA kits. Body mass index (BMI) (Kg/m^2) of post Ramadan overweight and obese male subjects exhibited considerable reduction ($p < 0.05$; $p < 0.05$) when compared to their respective pre Ramadan subjects. Noticeable and significant reduction was observed in BMI of post-obese females ($p < 0.05$). Plasma adiponectin ($\mu\text{g/ml}$) level of PRCM and PsRCM were 19.66 ± 5.47 versus 25.53 ± 6.65 , while PROM and PsROM were 13.90 ± 5.04 and 18.90 ± 5.85 , and average plasma adiponectin levels of PRObM and PsRObM were (12.76 ± 2.48 and 16.83 ± 4.68) respectively. Plasma TNF- α (pg/ml) level of PRCM and PsRCM were averaged at 19.66 ± 6.07 and 17.13 ± 5.36 respectively. In overweight PROM and PsROM average TNF- α concentration were 25.36 ± 10.34 and 22.16 ± 9.30 respectively. While in obese males were 36.08 ± 10.71 and 30.20 ± 10.17 . Post Ramadan Overweight Males ($P < 0.05$) and Post Ramadan Obese Males (PsRObM) ($P < 0.001$) exhibited significantly elevated plasma adiponectin ($\mu\text{g/ml}$) values. While plasma adiponectin mean concentration of only obese females were significantly improved at last week of Ramadan ($P < 0.01$). Mean Plasma adiponectin level of control females was 25.40 ± 5.19 and 26.60 ± 5.27 , while those of overweight females were 18.00 ± 6.58 and 23.50 ± 6.32 . Obese females have 14.06 ± 4.01 and 17.36 ± 4.46 $\mu\text{g/ml}$ of Adiponectin level. Plasma TNF- α level of PRCF and PsRCF was 20.38 ± 3.21 and 19.86 ± 3.81 respectively. PROF and PsROF have 27.97 ± 8.12 and 24.50 ± 7.24 , while PRObF and PsRObF were 30.49 ± 7.98 and 27.17 ± 7.34 respectively. Fasting in Ramadan significantly decreased TNF- α (pg/ml) levels of PsRObM and post obese females than Pre Ramadan groups ($P < 0.05$; $P < 0.01$) respectively. According to the result, Ramadan fasting may increase the plasma adiponectin level and decrease the TNF- α levels as well as body weight. In this regard, further investigations on larger sample sizes could focus on mechanism of enhanced adiponectin and reduced TNF- α in obese and overweight persons who fast during Ramadan. Also the possible association of dietary restrictions and weight loss should be included.

LIPID PROFILE AND CORRELATION TO HYPERTENSION

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Current study was performed on convenient fifty male and female volunteers (patient of Hypertension and High cholesterol). During this appraisal a human blood samples were collected from different areas, hospitals and laboratories of Karachi. The blood samples were used for the estimation of serum lipid parameters such as total cholesterol, triglycerides level, High-density lipoprotein cholesterol and Low-density lipoprotein.

One way variance analysis (ANOVA) statics was perform on SPSS version 22 which conclude that on average TC, TG, LDL-C and HDL-C are equal or not in all age categories. When age increase by 1 year then average logit value of TC, TG and LDL-C increase by 0.042, 0.000 and 0.071 and odd ratio not in favor of high lipid parameter and incline as 4.3%, 1.00% and 7.4% respectively and average logit of low HDL-C increase as 0.019 and odd ratio is 1.9%. Similarly average logit value of TC, TG and HDL-C decrease as 0.030, 0.001 and 0.067 when systolic blood pressure increase by 1mmHg and odd ratio of these dependent variables goes in favor of TC, TG and HDL-C while LDL-C increases as 0.002 with increase in SBP and odd ratio not in support of LDL-C. In the same way DBP increase to 1mmHg the average logit value of high TC and LDL-C is increased by 0.034 and 0.015 and also HDL-C in male individuals increases as 0.071 and odd ratios goes not in favor of TC, LDL-C and HDL-C parameters. However TG decrease as 0.001 by increasing DBP and odd ratio supports high TG. Result represent the average probabilities of TC level is 76%, TG 86%, LDL-C is 62% of all individual that shows strong association to CVD but HDL-C probability in male is 34% and in female is 55% decline (counter affect).

EFFICACY OF ESTROUS SYNCHRONIZATION PROTOCOLS THROUGH ESTROUS RESPONSE AND FERTILITY RATE IN NON-DESCRIPT COWS AND HEIFERS OF AZAD JAMMU AND KASHMIR DURING NON-BREEDING AND BREEDING SEASONS

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The objective of the present study was to compare the efficacy of estrous synchronization protocols through estrous response and fertility rate in non-descript cattle of Azad Jammu and Kashmir during non-breeding and breeding seasons. A total of 312 non-descript cows and heifers with body condition score ranges between 3.0 to 3.5 were randomly assigned to receive Ovsynch, CIDR alone and CO-Synch+CIDR protocols. Animals were inseminated at 16 h after second GnRH injection in Ovsynch, and 48 h after CIDR removal in CIDR inserted groups. The results revealed that during non-breeding season, the Ovsynch protocol was slightly better in terms of inducing heat and enhanced the conception rate (CR) in indigenous cows under farm (87.5%) and field (50.0%) conditions whereas, in case of non-descript heifers, CO-Synch+CIDR protocol enhanced the CR at timed artificial insemination (TAI; $P > 0.05$) under farm (50.0%) and field (33.33%) conditions. During breeding season, CR/TAI was similar (47%) in all groups of non-descript cows under field conditions whereas, CO-Synch+CIDR treatment yielded the satisfactory ($P < 0.05$) conception rates under farm conditions compared to field conditions in non-descript heifers. It is concluded that treatment of cows and heifers with estrous induction protocols will yield acceptable pregnancy rates and thus improve the reproductive performance that can faster the grading up or genetic improvement through AI during non-breeding and breeding season.

EFFECT OF OVSYNCH ESTRUS SYNCHRONIZATION PROTOCOL ON ESTRUS LENGTH AND INTENSITY IN NON-DESCRIPT INDIGENOUS (*BOS INDICUS*) CATTLE OF AZAD JAMMU AND KASHMIR

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For the rapid genetic improvement of non-descript indigenous (*Bos indicus*) cattle of Azad Jammu and Kashmir through artificial insemination (AI) both in natural and induced AI program heat detection is a key.

The study was undertaken to investigate the effect of ovsynch protocol on duration and intensity of natural and induced estrus in indigenous heifers and cows. In total of 12 heifers and 18 cows, estrus was synchronized using Ovsynch protocols. In natural estrus group 8 heifers and 6 cows were utilized in this study. They were observed for total estrus length and length of standing heat. The primary and secondary estrus signs of heat were observed. The total duration of heat in heifers was 18.63 ± 1.03 and 18.00 ± 1.58 hours in induced and natural group respectively ($P > 0.05$). The duration of standing heat in induced and natural estrus group of heifers was 10.63 ± 0.60 and 8.25 ± 0.73 hours respectively ($P < 0.05$). The total duration of heat in cows was similar between induced and natural group ($P < 0.05$) whereas the duration standing heat was longer in induced group (9.69 ± 0.62 h) than natural (6.66 ± 0.80 h) group ($P < 0.05$). The heat intensity score was not effected significantly ($P > 0.05$) in both heifers and cows between induced heat and natural heat. The duration of standing heat in indigenous heifers and cows has been shortened in natural heat compared to induced heat.

EFFICACY OF ESTROUS SYNCHRONIZATION PROTOCOLS THROUGH ESTROUS RESPONSE AND FERTILITY RATE IN NON-DESCRIPT COWS AND HEIFERS OF AZAD JAMMU AND KASHMIR DURING NON-BREEDING AND BREEDING SEASONS

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LEVEL OF HB CONCENTRATION IN SCHOOL GOING CHILDREN IN DISTRICT SWABI

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Hemoglobin is metalloprotein found in red platelets of greater part of vertebrates and its part is transporting of oxygen from metastasis organs of vertebrates to tissues. The customary measure of hemoglobin in youngsters is 11 to 16 g/dl. At the point when the value estimation of hemoglobin is diminishes than its conventional incentive with in the blood it brings about paleness. Press insufficiency iron deficiency might be a noteworthy wellbeing hazard all through the world. 65% Children had IDA in Pakistan though elective investigations unconcealed 70 to 78%. This investigation was dispensed to search out the measure of hemoglobin in school going children in District Swabi. This investigation was dispensed in four groups of District Swabi that are Swabi, Lahor, Razzar, Topi to search out level of hemoglobin in school going children. A total of 400 children were tried in our blessing study out of which 229 children were anemic and 171 children were non-anemic with offer of 57.25% and 42.75% severally. Our blessing study demonstrates that in Cluster

Swabi 50/100 children were anemic and 50/100 were non-anemic and in Cluster Lahor anemic children were 60/100 and 40/100 were non-anemic. Our blessing study uncovered that in Cluster Razzar the anemic children were 62/100 and non-anemic were 38/100 though in Cluster Topi anemic children were 57/100 and non-anemic were 43/100. From our examination it has been finished that iron deficiency attributable to low level of hemoprotein in blood at school going children keeps on being a huge downside in District Swabi furthermore as inside the whole nation. In this way mindfulness should be made by government furthermore as by wellbeing office in people with respect to the eating routine of school going children in order to diminish the quantitative connection of anemia in District Swabi.

CORRELATIONS BETWEEN PROINFLAMMATORY CYTOKINES AND ADIPONECTIN IN OVERWEIGHT PAKISTANI YOUTH

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Prevalence of overweight and obesity has increased numerous folds and majority of Asian nations are currently facing an epidemic of diseases associated with obesity. Cytokines are secreted in excess in the presence of adipose tissue and this intensifies the correlation between obesity and inflammation. The present study investigated the relationship between serum Adiponectin level and proinflammatory cytokines in 300 subjects of 17 to 30 years. The serum levels of glucose and lipids were determined by Chemistry Analyzer. Circulating levels of inflammatory markers including, adiponectin, C Reactive Protein, Interleukin 6 were measured by using an enzyme-linked Immunosorbent assay (ELISA). Adiponectin was significantly associated with Leptin in normal weight and obese II subjects ($p < 0.01$). Adiponectin had a significant negative correlation with CRP ($r = -0.324$, $p < 0.01$ in obese II group while, it revealed non significant relationship with Resistin and IL6 ($p > 0.05$) in all BMI groups. Adipokine and cytokine dysregulation affects through a control mechanism mediated by activation of anti-inflammatory pathways.

COMPARATIVE EFFECTS OF NEONATAL EXPOSURE OF FEMALE SPRAGUE DAWLEY RATS BISPHENOL A AND ITS ANALOGUE BISPHENOL S ON PUBERTAL DEVELOPMENT

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In the recent years, there has been growing concern about the endocrine disrupting potentials of bisphenol A, one of the most abundantly synthesized plasticizer. Due to the endocrine disrupting potential of bisphenol A, its use has been banned in many applications in several countries and has been replaced by other bisphenols. Bisphenol S has been introduced into the industry as a replacement of bisphenol A and has been used in several applications. It is considered more stable to temperature and insoluble in water, however, a trend of increasing concentrations of bisphenol S has been observed from 2010 to 2014 and its exposure to humans has also been increased. Double phenol rings in its structure makes it fit into estrogen receptor socket and it act like an estrogen to regulate estrogen receptor dependent pathways. Bisphenol A has been well documented for its endocrine disrupting potential however, very little is known about endocrine disrupting abilities of bisphenol S. Previously, exposure to bisphenol A during neonatal period of life resulted in Polycystic Ovarian Syndrome like symptoms in the adult hood. The present study aimed to compare the endocrine disrupting potentials of bisphenol S with bisphenol A, using female rats as an experimental model. On postnatal day 1 (PND 1) female pups born were randomly marked and were assigned for seven different treatments. Control group received subcutaneous injection of castor oil (50 μ L) subcutaneously from PND 1 to PND 10. Three groups of female pups were injected subcutaneously with different concentrations (0.5, 5 and 50 mg/kg) of bisphenol S while, the remaining three groups were subcutaneously exposed to (0.5, 5 and 50 mg/kg) bisphenol A. Body weight gain, onset of puberty and estrous cyclicity were monitored in all groups and were dissected in adult age on the

morning of estrous phase of cycle. Weight of reproductive organs was recorded after dissection. Plasma progesterone, estradiol and testosterone were determined through enzyme linked immunoassay while ovarian histology was performed for the histological alterations. Significant increase in final body weight was observed in the highest dose treated groups of both bisphenol S and bisphenol A. Similarly, delay in puberty onset was observed in the highest dose treated groups as compared to the control. In the same way altered estrous cyclicity was observed in bisphenol S (5 and 50 mg/kg) treated groups similar to bisphenol A (5 and 50 mg/kg) treated groups. Gonadosomatic index, absolute and relative weight of uteri was significantly reduced in both 5 and 50 mg/kg bisphenol S and bisphenol A treated groups than control. Number of cystic follicles was significantly increased in the ovaries of 50 mg/kg bisphenol S and bisphenol A (5 and 50 mg/kg) treated groups. Plasma concentrations of testosterone and estradiol were significantly increased, while plasma progesterone concentrations were significantly reduced in the highest dose treated groups than control. The results of the present study suggest that neonatal exposure to higher concentrations of bisphenol S can lead to similar alterations as bisphenol A exposure in the reproductive tissues of female rats and can lead to an increase in the number of cystic follicles in the ovary. Further studies are recommended here to obtain complete picture of Polycystic Ovarian Syndrome like symptoms induced by bisphenol S in rats.

RESPONSES OF STRUCTURAL AND FUNCTIONAL QUALITY PARAMETERS OF BUFFALO BULL SPERMATOZOA TO HYDROGEN PEROXIDE

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This study was carried out to investigate the effects of hydrogen peroxide (H₂O₂) on semen quality parameters with aim to validate them for buffalo bull. Qualifying ejaculates (motility > 60 %, concentrations, > 0.5 x 10⁹/mL and volume > 1 mL) from five buffalo bulls were diluted in PBS-0.1% BSA extender containing 10 µM H₂O₂, followed by assessment of computer-aided sperm motion analysis (CASA), subjective motility (SM, %), supra-vital plasma membrane integrity (SV-PMI, %), viability/ mitochondrial transmembrane potential (V/HP, %), acrosome integrity (V/IACR, %) total antioxidant capacity (TAC, µM/L) and lipid peroxidation (LPO, µM/mL) at different incubation times (0 min, 30 min, 60 min, 90 min and 150 min). Analyses of variance showed that H₂O₂ at a dose of 10 µM significantly decreased (P < 0.05) sperm progressive motility (PM, %) rapid velocity (RV, %) average path velocity (VAP, µm/s), straight line velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s), SM, SV-PMI, V/HP, V/IACR and TAC, whereas increased LPO in a time dependent manner than control. Moreover, exposure of H₂O₂ significantly increased DNA fragmentation in a dose dependent manner. Linear regression analysis showed a strong relationship between percent decline in PM and SM, SV-PMI, V/HP, V/IACR at different incubation times (R²= 0.944, P < 0.01, R²= 0.875, P < 0.05, R²= 0.941, P < 0.01, R²= 0.791, P < 0.05, respectively). We concluded that sperm quality of buffalo bull was negatively modulated by H₂O₂ and may be used for monitoring the effectiveness of semen quality.

SURVEY OF HYPOTHYROIDISM IN FERTILE FEMALES IN HYDERABAD AND JAMSHORO DISTRICTS OF SINDH

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Thyroid gland is situated in the anterior (front) neck below the skin and muscle layers. The thyroid gland is shaped like a butterfly with two wings being represented by the left and right thyroid lobes which wrap

around the trachea. Thyroid gland secrete hormones T3 and T4 which are responsible for metabolism. The low secretion of thyroid hormone which are cause of hypothyroidism. Hypothyroidism is common metabolic disorder which is resulting from insufficient or too little secretion of thyroid hormones. The purpose of this study was estimate the ratio of different age group of infertile female patient with suffering from hypothyroidism was investigated in different hospitals of Hyderabad from March 2015 to feb 2016. A total number of hypothyroidism patients 110 of females unlike age groups (20-30, 31-40) were analyzed during study period. Out of 110 patients 85 infertile females were observed during present study. Hypothyroidism infertile females was common in all age groups. The laboratory investigations include the whole thyroid profile TSH, T3 and T4. Serum Aliquots technique was used. On the basis of primary data all patients were suffering from hypothyroidism infertile females. The data also indicated that the occurrence of symptoms of most of the patients were similar fatigued easier 81%, swelling of face 68%, muscle weakness 45%, mental confusion 83%, high cholesterol level 46%, increase heart rate 51%, menstrual disturbance 65%, swelled neck 98%, increase sweating 85%, excessive appetite 75%. Our results showed the most of the patients were unaware of hypothyroidism or thyroid diseases which is cause of infertility.

RELATIONSHIP OF INFERTILITY WITH POLYCYSTIC OVARIAN SYNDROME (PCOS) AND OBESITY IN SPECIFIC FEMALE POPULATION OF KARACHI PAKISTAN

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A study was carried out to evaluate the correlation of infertility with PCOs, LH, FSH, Prolactin and obesity in specific population of Karachi from May 2012 to June 2014. Hundred infertile women under the age of 42, were tested for Polycystic Ovarian Syndrome (PCOs). Different physiological factors (systolic and diastolic blood pressure) associated with infertility were also assessed. It is attributed by the study that 43% of the subjects had PCOs among 100 infertile females of Karachi city in which 48.83% of the cases were obese. A significant correlation ($P = 0.01$) was observed between weight and PCOs. Levels of FSH (8.68 ± 1.23), LH (16.04 ± 0.99) and prolactin (20.75 ± 1.46) were also investigated. LH and weight were found to have significant correlation ($P = 0.01$) between them. This research is an excellent contribution in the field of medical as well as biological sciences. The study shows many future perspectives for future research.

HORMONAL FLUCTUATIONS AS POTENTIAL INDICATORS OF GESTATIONAL DIABETES MELLITUS (GDM)

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Gestational diabetes mellitus (GDM) is recognized as an imbalance between insulin resistance and insulin secretion, leading to maternal hyperglycemia. GDM has common features with type 2 diabetes mellitus; there are similar risk factors, mechanisms (i.e. insulin resistance and impaired insulin secretion) and genetic susceptibility for the both disorders. The major factors contributing to GDM are the placental hormones, such as human placental lactogen, progesterone, cortisol, growth hormone and prolactin. Biochemical analyses of blood serum in pregnant women with GDM is hypothesized to help understand the pathogenesis of the disease and also the discovery of candid biomarkers. The present proposal was, therefore, designed in an attempt to achieve the said objectives. Antenatal pregnant women ($n = 300$) were selected for blood samples as a case control study including 176 with positive clinical/family history and 124 with negative clinical/family history during the early second trimester (14–18 weeks of gestation). All the subjects were followed up to the early third trimester (24–28 weeks of gestation) for second sampling until the onset of GDM. In hormonal profile, mean values of tissue plasminogen activator (TPA), progesterone and cortisol concentration were significantly higher ($p < 0.05$) and mean vitamin D and sex hormone binding globulin (SHBG) were significantly lower in early second trimester.

in those patients who subsequently developed GDM in late third trimester as compared to those who didn't develop GDM. Maximum number of GDM developed cases were those who were having positive clinical/family history. These parameters may be used to detect early onset of GDM and for prevention of the disease, or to identify women susceptible to develop GDM. Early diagnosis of gestational diabetes will decrease adverse neonatal and maternal outcomes. In conclusion, we have shown that early prediction of GDM is possible. Among the biomarkers tested, each of vitamin D, TPA, cortisol, progesterone and SHBG has a high sensitivity in predicting cases with GDM. An overall comparison of hormonal profile in negative history group (n=124) and positive history group (n= 176) during early second trimester (14-18 weeks) and early third trimester (24-28 weeks) of gestation is tabulated below.

Parameters	Early Second Trimester		Early Third Trimester	
	Negative History	Positive History	Negative History	Positive History
Vitamin D(ng/mL)	43.44±0.79 ^a	40.49±0.99 ^b	21.18±0.73 ^c	30.66±0.73 ^d
SHBG(nmol/L)	210.39±5.97 ^a	167.44±5.05 ^b	105.65±2.24 ^c	114.07±2.63 ^c
Cortisol(nmol/L)	207.15±4.45 ^b	224.05±6.39 ^b	323.35±4.25 ^a	319.02±6.92 ^a
TPA(ng/mL)	5.71±0.13 ^d	6.67±0.09 ^c	8.34±0.13 ^b	9.58±0.15 ^a
Progesterone(ng/mL)	52.23±0.63 ^d	56.77±0.47 ^c	66.35±0.64 ^b	69.11±0.82 ^a
Different alphabets (a,b,c,d) on means±SEM show significant differences at p<0.05; Order of significance is as: a>b>c>d				

A COMPREHENSIVE ANALYSIS OF THYROID AND PROTEIN PROFILING IN OVERWEIGHT AND OBESE YOUNG ADULTS OF URBAN POPULATION OF PUNJAB, PAKISTAN

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Obesity is a major health hazard and it is becoming a threat in Pakistan. More than one billion adults all over the world fall in category of excess weight. Increasing trend of overweight and obesity has been reported in both developed and developing countries. According to WHO, BMI greater than 25kg/m² is considered overweight and above 30 kg/m² is considered obese. The study aims to determine the effect of obesity on thyroid and protein profile in overweight and young obese adults. For this purpose, blood samples of 60 controls, 35 overweight and 40 obese individuals were selected according to their BMI. Anthropometric parameters including hip circumference, waist circumference, waist to hip ratio (WHR), waist to height ratio (WHtR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate were determined. Clinical chemistry analyzer and ELISA were used for quantitative determination. One way ANOVA was employed to access the variations among groups. In the present study, significant (p<0.001) elevation of BMI was observed in overweight and obese group. Anthropometric parameters also showed highly significant increase when compared with control. A significantly (p<0.001) increasing trend of T₃ and TSH was found in obese group. A highly significant decrease (p<0.001) was observed in serum albumin level in overweight and obese groups in comparison with controls. This decrease might be due to decreased albumin synthesis from hepatic congestion in obese individuals. An increasing trend was observed in serum globulin level in obese as compared to controls, due to increased immunoglobulins production. Higher globulins level and low albumin level counter balance the total protein level in the serum. Conclusively, increased weight and obesity can lead to many long and short term lethal metabolic deviations on health. Obesity is carrying enough body fat to put individuals at risk of multiple disorders including hypertension and cardiovascular diseases. Therefore, it is recommended that increasing physical activity and improving eating habits not only inhibits the onset of obesity but also the subsequent risks of other metabolic diseases.

**GREEN SYNTHESIS OF GOLD NANOPARTICLES FROM *CARICA PAPAYA* LEAVES EXTRACT
AND EVALUATION OF THEIR ANTIMICROBIAL, ANTIOXIDANT AND
HEPATOPROTECTIVE POTENTIALS**

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In recent years, gold nanoparticles (AuNPs) prepared by medicinal plants have become the focus of much attention in biomedical research especially in the content of nanomedicine due to their distinctive physicochemical properties. The purpose of this study was to investigate the antimicrobial, antioxidant and hepatoprotective potential of gold nanoparticles synthesized from leaves extract of *Carica papaya*. The synthesized nanoparticles were characterized by UV-visible, FTIR, XRD and SEM and then antimicrobial and antioxidant activities of AuNPs were evaluated in vitro. Finally, hepatoprotective potential of AuNPs was checked against carbon tetrachloride (CCl₄)-induced liver damage in rabbits. The rabbits were randomly divided into two groups (n=6) control group received only normal saline while treatment group received AuNPs @ 0.1 mg/kg b. wt/day orally for 14 days. After 14 days, rabbits were sacrificed and blood samples collected and evaluated for serum biochemical parameters ALP, ALT, AST and bilirubin and liver were excised for histopathological alterations. The results showed successful preparation of good quality AuNPs from extract of leaves of *Carica papaya* confirmed from SEM, FTIR, UV-Vis and XRD. Gold nanoparticles showed significant antimicrobial and antioxidant activities. AuNPs showed hepatoprotective activity as treatment group showed reduced values of ALP (66±4.4), ALT (103±9.1), AST (81±5.9) and Bilirubin (0.81±0.1) as compared to control group. Moreover, histopathological analysis confirmed improvement in liver structure of treatment group as compared to control group. These study suggest that AuNPs synthesized from leaves extract of *Carica papaya* have hepato-protective effect along with good antioxidant and antimicrobial potential.

CLINICOPATHOLOGICAL PATTERNS OF UTERINE LEIOMYOMA PATIENTS

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Uterine leiomyomas are highly frequent gynecological neoplasms with major public health impact. The objective of study is to systematically analyze clinico-pathological patterns, electrolyte profile and marker enzymes in leiomyoma patients. The study comprised of 44 control subjects and 46 leiomyoma patients, aged 21- 50 years. Clinicopathological findings, anthropometric parameters and detailed history of disease were recorded through a standardized questionnaire followed by uterine ultrasound examination. Blood samples were drawn for the measurement of serum electrolytes and marker enzymes levels by using commercially available kits. Among clinico-pathological patterns, high frequency of leiomyoma (43.5%) was found in the age between 30-40 years. Intramural uterine leiomyomas were more common type of leiomyoma (61%) and majority of leiomyoma were found single (52%). Menorrhagia was common clinical manifestation with 63% leiomyoma cases. Family history of fibroid was observed in 26% leiomyoma women. A significant increase in body mass index (BMI), diastolic blood pressure (DBP) and significant decrease in parity was recorded in leiomyoma subjects in comparison with controls. Serum electrolytes analysis revealed a significant increase in the concentrations of chloride (Cl⁻¹) as well as calcium (Ca⁺²) and significant decrease in potassium (K⁺¹) concentration in leiomyoma group when compared to the controls. While, non-significant difference was recorded in serum sodium (Na⁺¹) level between comparable groups. Results of marker enzymes analysis manifested significant elevations in the serum levels of acid phosphatase (ACP), aspartate transaminase (AST) and alanine transaminase (ALT) in leiomyoma patients in relation to controls. While, non-significant variations were found in serum alkaline phosphatase (ALP) levels between comparable groups. Elevated serum Na⁺¹, Cl⁻¹ and Ca⁺² and reduced K⁺¹ concentrations in leiomyoma patients characterize increased estrogen level, responsible for leiomyoma growth and serum levels of ALT, ACP and AST are viable diagnostic markers of uterine leiomyoma.

STUDIES ON THE ENDOCRINE AND METABOLIC CORRELATES OF POLYCYSTIC OVARY SYNDROME IN DISTRICT RAHIM YAR KHAN

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Polycystic ovary syndrome (PCOS) has become the major gynecological problem throughout the world. It is the result of an endocrine disorder. An attempt was made to study the female individuals confronting PCOS at district Rahim Yar Khan. In this regard three hospitals i.e. Sheikh Zaid, Al- Saeed complex and Salman Medicare were visited in order to examine the female patients belonging rural population suffering from the irregularities in their menstrual cycle. In all 46 patients were examined: all having the PCOS. From the patients examined 10 patients were suffering from the oligomenorrhea, 27 were of dysmenorrhea and only two were suffering from Amenorrhea. The Mean age of the patients suffering from oligomenorrhea was 29.6 and mean standard error was 2.35. The mean weight of the patients of oligomenorrhea was 56.0, whereas mean standard error was 2.37. The mean age of dysmenorrhea was 30.74 and the mean standard error was 1.98, however the mean weight of dysmenorrhea was 56.44 and the mean standard error was 2.22. The mean age of the patients of amenorrhea was 38.5 and mean standard error was 8.15, the mean weight of amenorrhea was 72.5 and the mean standard error was 1.77. It is concluded from the studies carried out that maximum patients were suffering from the disorder dysmenorrheal probably because of lack of exercise and some possible obstruction in the uterine wall / adenomyosis.

INCIDENCE OF ENDOMETRIOSIS AND INFERTILITY IN POPULATION OF MULTAN

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The incidence of endometriosis and infertility from Nishter hospital, and different hospitals of Multan city was assessed. The data was collected from 332 females and 178 males. Out of these in case of males 114 were control whereas 64 were afflicted with infertility, whereas in case of females out of 332, only 71 (22.36%) were afflicted with endometriosis and 136 showed infertility without endometriosis. The subjects were distributed in to 4 different age groups such as ≤ 25 , ≤ 30 , ≤ 35 , ≤ 40 years. The incidence of endometriosis was highest (38.88%) in ≤ 30 group and lowest (9.73%) in ≤ 35 years and different age groups were significantly different ($p=0.001$). The incidence of infertility showed that it was significantly different ($P<0.05$) in males (35.6%) when compared with females (64.4%). The data regarding infertility was divided into 5 age groups as ≤ 25 , ≤ 30 , ≤ 35 , ≤ 40 , ≤ 45 years. The incidence of infertility was highest (37.8%) in ≤ 30 age group and lowest in (2.8%) in ≤ 45 age group and it was significantly different ($P=0.000$).

BISPHENOL A; A XENOESTROGEN CAUSES SMALL BOWEL CALCIFICATION AND DISRUPTION IN INTESTINAL TRACE ELEMENTS

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BPA, a xenoestrogen, is one of the contaminants of emerging concern and causes many serious health implications in humans. The current study was aimed at investigating the deformities of structure that arise by BPA exposure to small intestine along with tissues trace elements estimation as well as study of serum profile and hepatic gene expression involved in inflammatory response against BPA. Two major groups of *Rattus norvegicus* were established: one control and other experimental group which was divided into four groups based on dose (10 mg/kg/bodyweight and 25 mg/kg/bodyweight respectively) and duration of exposure (6 and

12 weeks respectively). Histological study of small intestine showed the distorted structures in experimental groups. The special staining performed, illustrated the accumulation of calcium deposits in the small intestinal tissue in treated groups. Trace metal estimation showed a significant increase in the metallic content of sodium and iron and a decrease in calcium content in experimental groups ($p=0.05$). Serum profiling illustrated an increase in Total Iron Binding Capacity and glucose levels and a decrease in serum Total iron level ($p=0.05$). Gene expression study of proinflammatory cytokine (IFN- α) illustrated a significant increase in its expression in experimental groups i.e. L6 (0.63 fold \pm 0.030), H6 (0.68 fold \pm 0.003), L12 (0.71fold \pm 0.014) with the highest level in the H12 group (1.02 fold \pm 0.066). From all these findings, it can be inferred that BPA caused many structural alterations in the small intestinal tissue which further affected its functioning. The calcium deposits seen through special staining affected the motility of small intestine and caused its dysfunction. It was also induced that BPA affected the homeostasis of iron and glucose and caused its imbalance after serum profiling. Furthermore, the genetic expression studies showed that BPA leads to the high inflammatory chronic response by cytokines.

EFFECTS OF Cr₃O₄ NANOPARTICLES ON HEMATO-IMMUNOLOGICAL INDICES OF *LABEO ROHITA*

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Fish is an important component of human diet and one of the cheapest sources of quality animal protein. Nanoparticles (NPs) have numerous applications in nano-biotechnology. However, apart from its many beneficial roles nanoparticles have also been reported for their toxic effects. In the present study Cr₃O₄ NPs were used to determine their effects on hematological, immunological and histological indices of Indian major carp *Labeo rohita* (juvenile). Juvenile *Labeo rohita* was fed with basal diets and basal diets supplemented with Cr₃O₄ NPs to triplate groups of fish. The survival rate of Cr₃O₄ NPs treated group was low as compared to control group. Cr₃O₄ NPs caused a significant decrease in Total Erythrocyte Count (TEC) in treated group. Total protein analysis revealed a significant increase ($p < 0.05$) in the Cr₃O₄ NPs treated group over the control group. A decrease in Total Leucocyte Count (TLC) was observed in Cr₃O₄ NPs treated group in comparison to the control group. The morphology of the red blood cells also revealed that the Cr₃O₄ NPs reduced the surface area of blood cells. Our results demonstrated that Cr₃O₄ NPs in aquatic environment induced hematological, immunological and histological indices of *Labeo rohita*.

CRYOPRESERVATION OF INDIAN RED JUNGLE (*GALLUS GALLUS*) SEMEN WITH ETHYLENE GLYCOL

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Ethylene glycol has cryoprotective and antioxidant properties and may ameliorate the injuries to sperm during the stressful process of cryopreservation. Cryopreservation of Indian red jungle fowl sperm with glycerol resulted in substantial damages. Therefore, present study was designed to explore the beneficial effects of ethylene glycol on Indian red jungle fowl sperm during cryopreservation. For this purpose, semen was collected from nine mature cocks and initially evaluated for volume, motility and concentration. The ejaculates with >60% motility were cryopreserved with 0% (control; 20% glycerol), 5%, 10%, 15% and 20% ethylene glycol. Semen quality and biochemical parameters viz; motility, plasma membrane integrity (hypo-osmotic swelling test), viability (lake glutamate staining), acrosome integrity (giemsa staining), chromatin damage (aniline blue staining), free radical scavenging activity (DPPH assay), lipid peroxidation (malondialdehyde level), and metabolic activity (MTT reduction assay) were assessed at post-dilution, cooling, equilibration and freeze-

thawing. Sperm motility, plasma membrane integrity, viability, acrosome integrity was recorded highest ($P<0.05$) with 20% ethylene glycol compared to other experimental extenders and control during all stages of cryopreservation. Furthermore, least ($P<0.05$) chromatin damage was recorded with 20% ethylene glycol. Free radical scavenging activity was increased ($P<0.05$) after dilution, cooling, equilibration and freeze-thawing. The malondialdehyde level in sperm and seminal plasma increased ($P<0.05$) after each stage of cryopreservation. The results of MTT reduction test to reduce tetrazolium salt (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to formazan were comparable with sperm viability and extender having 20% ethylene glycol showed higher ($P<0.05$) percentage of metabolically active sperm cells compared to control. It is concluded that 20% ethylene glycol has the ability to protect the Indian red jungle fowl sperm from cryodamages and oxidative stress.

CORRELATION OF ELECTROLYTES BETWEEN DRINKING WATER AND BLOOD AMONG HYPERTENSIVE PATIENTS IN SARGODHA

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During the present study, the correlation between electrolytes, present in drinking water and serum of hypertensive patients in Sargodha was studied. The samples from District Mandi Baha-Ud-Din were taken as controls. The level of sodium, potassium, calcium, and magnesium was significantly high in drinking water in Sargodha. Similarly, the level of these electrolytes was significantly higher in the serum of hypertensive patients in comparison to control. A positive correlation was observed in both drinking water and serum electrolytes in the study group. This excessively high level of studied electrolytes may be a risk factor for this group and generally healthy people. It may also have a role in developing and sustaining hypertension in the study group because they also cause a change in body fluid and kidney may be unable to reduce them due to their high level. Further studies on the role of electrolytes, other than Na, in hypertension are suggested to be carried out in this area.

INVESTIGATION OF PHOSPHORYLATED IRS-1 AND -2 IN DEVELOPMENT OF TYPE II DIABETES

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Deficiency of insulin causes insulin resistance that leads to type I and II Diabetes Mellitus. The binding of insulin with insulin receptor and activation of insulin receptor substrates by auto-phosphorylation of tyrosine residues initiates downstream pathways that are mainly and tightly regulated by post-translational modifications on serine, threonine and tyrosine residues. *O*-glycosylation, an equally important and abundant modification, has shown to inhibit phosphorylation on the same or neighboring serine and threonine residues, such sites are called Yin Yang sites. The two insulin receptor substrates produced in humans are IRS-1 and IRS-2. In this study, by using different bioinformatics tools, 37 potential Yin Yang sites and 146 predicted Ser/Thr and Tyr phosphorylation sites were predicted for IRS-1, while 146 phosphorylation and 57 glycosylated sites were predicted for IRS-2. Furthermore, using SDS PAGE and indirect ELISA, a quantitative analysis of IRS-1 protein was determined in both types of diabetes and the role of phosphorylation at Serine 1101 in IRS-1 and 1149 in IRS-2 was determined which suggested that phosphorylation at these sites are associated with Type II Diabetes.

SEX DETERMINATION BY NEUTROPHILS DRUMSTICKS IN HUMANS AND DOGS**Mushtaq Hussain Lashari, Rabia Hameed, Nuzhat Sial, Umer Farooq and Muhammad Saleem Akhtar***Department of Life Sciences, The Islamia University of Bahawalpur, Pakistan*¹*University College of Veterinary & Animal Sciences, The Islamia University of Bahawalpur*²*Department of Clinical Sciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan*

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The present study was conducted with an objective to determine the sex by neutrophil Barr bodies in humans and dogs. In humans (n=40), blood was collected aseptically from either the cephalic or the median cubital vein of each subject as per the directed guidelines of the World Health Organization. Regarding dogs (n=30), the blood was collected from saphenous vein after proper restraining. The blood was collected in lavender-top colored vacutainers (EDTA) for preservation till processed in next 08 hrs. Thin blood smears were made and stained using Field Stain 'A' and 'B'. Neutrophils were examined from blood smears obtained with informed consent from healthy females and healthy males. Only neutrophils with identifiable terminal lobes were examined. The mean value \pm SE of the Barr body were calculated and difference and males and females of both species were analyzed through independent t-test. The number of Barr bodies were significantly ($P \leq 0.05$) higher in females as compared to males in case of humans. However, there was no significant difference between male and female dogs regarding the presence of Barr bodies. The results of the present study revealed that sex chromatin investigations might prove to be of direct clinical value for the early detection of sex. This is the baseline data regarding the subject matter and requires a large number of populations in variable geographical location for future studies.

TRIMESTER BASED IRON DETERMINATION AMONG PREGNANT WOMEN**Aisha Nawaz***Lahore College for Women University Lahore Pakistan*

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Pregnancy is one of the most important periods in life when increased micronutrients and macronutrients are most needed by the body both for the health and well-being of the growing fetus as well as mother. The purpose of this study was to determine prevalence of anemia among pregnant women registered for antenatal care at the rural Health Centre of region Kasoor, Punjab, Pakistan. This cross sectional study was designed to assess the iron level in the blood of pregnant ladies of first and third trimester. The data was collected during the months of November 2016 to March 2017 by taking the patient's blood samples and using questionnaires. Sample size of 50 patients for this study was estimated and the sample was selected using simple random sampling method. Blood samples of 50 pregnant ladies of age 20-40 years were collected from Kasoor region. Among them 25 ladies were of 1st trimester and 25 were of 3rd trimester. The data was collected from a private hospital of District Kasoor and were tested in the laboratory. The questionnaires were filled by the women to assess their nutritional status and other medical complications (blood pressure, miscarriage, and anemia) during or before pregnancy to know about the women's overall health status and other conditions associated to that like nature of anemia, pattern of menstruation cycle. Iron level of the pregnant ladies was measured using their hemoglobin. The experiment was carried out by using special calorimeter and spectrometer. The data was compiled in the form of tables, graphs and percentage. The results showed that more than half (76%) of the pregnant women of 1st trimester had mild anemia and 88% women of 3rd trimester were anemic and were iron deficit. Anemia was more prevalent among the patients in third trimester than patients who registered in first trimester. Prevalence of anemia in pregnancy in region Kasoor is high. Pregnancy care, iron and folic acid supplementation should be encouraged to reduce this problem. Early detection of cases and cause of anemia in this population is necessary and good treatment with antenatal care must also be encouraged.

CARDIOVASCULAR RISK ASSESSMENT IN AUTOMOBILE MECHANICS AND PETROL PUMP ATTENDANTS

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In order to investigate the effects of lead exposure on risk of cardiovascular disease, plasma cholesterol and its fractions as high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride were evaluated in various auto-mechanics (AM) and petrol station attendants (PSA) in Lahore, Pakistan. Cardiovascular risk factors were assessed in all study groups by measuring their anthropometric parameters as well as lipid profile. Total cholesterol, triglyceride, LDL, VLDL was significantly higher in AM and PSA groups, as compared to control group. On the contrary, HDL showed a non significant decrease in AM group. However, a significant decrease in HDL was observed in petrol station workers. Among anthropometric parameters, BMI showed statistically non-significant differences when control, AM and PSA groups were compared. Results indicate that increased plasma TC, TG, LDL and VLDL in the occupationally exposed auto-mechanics and petrol station attendants together with the borderline lower levels of HDL ultimately raises a lot of concern about their risk of cardiovascular disease. Among them, low level of high density lipoprotein is the leading cause of mortality from cardiovascular disease. Furthermore, lead exposure enhances cholesterol synthesis and transport to peripheral tissues whereas reverse cholesterol transport to the liver is not affected. Anthropometric parameters were not significantly different in experimental and control groups because the study comprises an active population, involved in the energy and manual based activities, so it is suggested that the workers engaged in some physical activity would be at lower risk of cardiovascular diseases. Since, several toxicants including lead are the leading cause of occupational health hazards, numerous protective measures should be taken to circumvent occupational exposure to such toxicant at workplace.

SHAPE OF NAIL SPECIALLY LUNULA IN DIABETIC, ASTHMATIC AND TUBERCULOSIS PATIENTS

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The aim of this study was to observe the change in nail shape especially lunula in asthmatic, diabetic and tuberculosis patients. This study was performed at Bahawal Victoria Hospital (BVH) , Punjab, Pakistan. The hospital was visited daily and studied the patients there from April 2016 to May 2016. Total 50 patients from each disease *i.e.* asthma, diabetes and tuberculosis were selected. The nail of different patients were observed and studied. There was also a control group. The control group contained healthy persons. Results were evaluated by comparing the groups with control group. It was observed that the persons having lunula on their nails are an indicator of good thyroid health and digestion. Such persons have very active immune system.

ESTIMATION OF CARBOHYDRATE (CORN) APPARENT DIGESTIBILITY IN *CIRRHINUS MRIGALA* (MORI) UNDER THE INFLUENCE OF INORGANIC CHROMIUM

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A feeding trial was conducted to determine the effect of inorganic dietary chromium supplementation on apparent nutrient digestibility coefficient (%) of gelatinized and non-gelatinized corn in *Cirrhinus mrigala* (*mori*) fingerlings for three months. By giving various levels of chromium chloride hexahydrate in six test diets

viz., CCH1 (G/0.0Cr₂Cl₃6H₂OmgKg⁻¹), CCH2(NG/0.0Cr₂Cl₃6H₂Omg Kg⁻¹), CCH3(G/0.2Cr₂Cl₃6H₂Omg Kg⁻¹),CCH4 (NG/0.2Cr₂Cl₃6H₂Omg Kg⁻¹),CCH5 (G/0.4Cr₂Cl₃6H₂Omg Kg⁻¹) and CCH6 (NG/0.4Cr₂Cl₃6H₂O mg Kg⁻¹) were prepared. Results showed highest apparent digestibility coefficient (ADC) of nutrients dry matter, crude lipid and gross energy in test diet CCH5 that was gelatinized and supplemented with chromium 0.4 mg/Kg while, for crude protein higher value of nutrient digestibility was recorded in CCH3 test diet (G/0.2, Cr₂Cl₃6H₂Omg/Kg). It was concluded that chromium supplementation with gelatinized corn in fish (*Cirrhinus mrigala*) diet can improve the nutrients digestibility more efficiently.

VARIATION IN HAEMOGRAM AFTER ACUTE ADMINISTRATION OF MONOSODIUM GLUTAMATE (MSG) IN MICE

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The present work was aimed to study acute phase response up to 24h with monosodium glutamate, one of the world's most extensive commercially used food additives. MSG (0.04g) was given intraperitoneally (Ip) to control (I) and experimental (II, III, IV&V) mice groups. Blood drawn was processed for haematological and transaminases analysis and livers were extracted after dissection at set time point (3, 6, 12, & 24). Control (I) was injected with pyrogen-free normal saline parallel to the experimental groups. Regarding haemogram all studied parameters shown statistically significant variations. RBC indices (HCT, MCV, Hb, RBC) shown decrease in their concentration in all experimental groups except group IV compared to control while MCH and MCHC depicted increment in all time points. Platelets exhibited thrombocytopenia while MPV shown rise in all experimental groups compared to control. WBC (TLC) exhibited leucopenia in all studied experimental groups compared to control. Regarding differential leucocyte count, monocyte reflected a major decline in their concentration compared to control while the rest leucocyte shown rise in their concentration. Taken together all the hemogram results it can be inferred that even low dose of MSG can alter the blood cell indices. Hence use of this taste enhancer should be avoided.

7. TOXICOLOGY

EFFECT OF USING 50 MG/KG AND 150 MG/KG OXYTETRACYCLINE IN FEED ON GROWTH AND HEMATOLOGY OF *LABEO ROHITA*

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The purpose of present study was to determine the effect of oxytetracycline on growth and hematology of *Labeorohita*. It was directed in glass aquaria having 80 liters capacity of water in Saline Fisheries Laboratory, Department of Zoology, Wildlife and Fisheries. Group A were considered as control group having 30% crude protein without oxytetracycline. Group B and Group C were nourished with 50 and 150mg/kg oxytetracycline @ 7% of body weight. After 8 weeks, the average total weight gain (g) in T₀ was 0.91±0.02g lower than T₁ 1.142±0.05g and T₂ 1.514±0.03g. The average total length gain (cm) in T₀ was 0.35±0.095cm while 0.64±0.181cm and 0.85±0.018cm in T₁ and T₂ respectively. After blood analysis the results showed that the concentration of RBC's (10⁶/μL) was 0.03±0.01 (10⁶/μL), 0.38±0.011 (10⁶/μL), and 0.8±0.111 (10⁶/μL) in T₀, T₁ and T₂ respectively. The WBC's (10³/μL) concentration in T₀ was 1.6± 0.01(10³/μL) while increased in T₁ 16±0.17 (10³/μL) and T₂ 35±0.19 (10³/μL). Platelets (10³/μL) concentration was decreased in T₀ 100±0.11 (10³/μL) as compared to T₁ 200±0.15 (10³/μL) and T₂ 625±0.18 (10³/μL). Hematocrit (%) concentration was increased in T₁ 2.02±0.001% and T₂ 2.39±0.001% as compared to control (T₀) 0.15±0.01%. Hemoglobin (g/dL) counts were decreased in T₁ 1.3±0.019 (g/dL) and T₂ 1.0±0.005 (g/dL) as compared to control (T₀) 2.0±0.02 (g/dL). MCV (fL) concentration was decreased in T₁ 42±0.21 (fL) and T₂ 38±0.21 (fL) as compared to control (T₀) 46±0.01 (fL). MCH (pg) counts were decreased in T₀ 25±0.01 (pg) as compared to T₁ 26±0.21 (pg) and T₂ 31±0.11 (pg). MCHC (g/dL) concentration was increased in T₁ 36±0.24 (g/dL) and T₂ 40±1.21 (g/dL) as compared to T₀ 33±0.05 (g/dL). After results it was concluded that when oxytetracycline inclusion in feed, it increased the growth of *Labeorohita* but also produced negative effects in the blood of *Labeorohita*.

PROTECTIVE EFFECT OF VITAMIN C ON GENTAMICIN INDUCED TOXICITY IN SPRAGUE-DAWLEY RATS

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Gentamicin is an aminoglycoside antibiotic, which is broadly used for the treatment of life-threatening Gram-negative infections. However, gentamicin administration for more than seven days may cause nephrotoxicity and oxidative stress. In the present study, we investigated the protective role of vitamin C against gentamicin-induced toxicity in albino rats. Adult male laboratory rats treated with Gentamicin (100 mg.kg⁻¹ bw, i.m.) were additionally treated with vitamin C (250 mg.kg⁻¹ b.w) orally. Appropriate controls were run in parallel. At completion of the experiments, venous blood was collected and body organs viz., brain, heart, lung, liver and kidneys were excised. Serum and tissue homogenates were prepared. Antioxidant enzymes (SOD and CAT) activity, reactive oxygen species ROS and lipid peroxidation through TBARS was carried out. Results were compared statistically at P<0.05. Results showed significant decrease in serum (*p<0.05), heart (*p<0.02), liver and kidney (*p<0.01) tissues ROS and TBARS levels in Vitamin C treated groups. In contrast, significant increase occurred in CAT activity in the serum (*p<0.02) and kidneys (**p<0.001) of Vitamin C treated groups. Similarly, significantly increased (*p<0.05) SOD activity was found in the serum, brain, lung, heart, liver and kidney tissues of treated rats in comparison with gentamicin treated control. Our current study concludes that Vitamin C effectively countered gentamicin-induced tissue oxidative stress via decreasing the free radicals and lipid peroxidation at one end, and increasing the antioxidant enzyme activities at other.

EVALUATION OF TOXIC EFFECTS OF FIPRONIL IN BIOCHEMICAL AND HISTOLOGICAL PARAMETERS OF LIVER OF ROHU (*LABEO ROHITA*)

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Fipronil is a phenylpyrazole a GABA receptor blocker insecticide is used to control insect pests like crickets, thrips, aphids, grasshoppers, beetles and weevils. Fipronil can be potentially toxic to aquatic invertebrates and vertebrates, if enters into fresh water bodies via the agricultural fields. Presently, rohu fish was exposed for 96 h to low (220µg/L) and high (350µg/L) doses of fipronil formulation. Control group was run alongside. At end of exposure, blood samples were collected via cardiac puncture; following this fish were dissected. For histomorphological analyses, liver tissues were excised. Liver enzymes the AST, ALT, ALP and, as well as albumin, bilirubin and glucose were determined. Results were compared with the control and between groups at $P < 0.05$. Fishes treated with fipronil at high dose (350 µg/L) had significantly increased concentrations of serum ALP, bilirubin and glucose ($p < 0.001$; $p < 0.001$; $p < 0.001$ respectively), while serum glucose levels significantly increased at experimental low dose (220µg/L) group ($p < 0.001$) and serum bilirubin levels significantly decreased at experimental low dose (220 µg/L) group ($p < 0.001$). No significant alteration was observed in serum AST, ALT and albumin concentrations in both treatment groups. Behavioral and histomorphological changes were also observed. Histomorphologically, liver tissue showed nuclear degeneration, eosinophilic granules, sinusoidal alterations and cellular hypertrophy, irregular shaped nuclei, congestion, nuclear degeneration and vacuolar formations. The present study demonstrates that fipronil is highly toxic to fish. These results indicate that liver biomarkers and histopathological change could be employed as potential biomarkers for observing pesticide residues existing in aquatic ecosystem and provides useful parameters for assessing physiological effects in fish. In future, further studies are required to be conducted to understand fish responses to toxicants.

AN INVESTIGATION ON MALE REPRODUCTION FOLLOWING EXPOSURE TO ACUTE AND SUB-ACUTE DOSES OF FIPRONIL TO LABORATORY RATS

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Fipronil is a broad-spectrum N-phenylpyrazole insecticide that kills insect pests via blocking the gamma-amino butyric acid (GABA) receptors. It is being used in agriculture and public health management since the mid-1990s. Fipronil is not present in the air generally due to being least volatile but drift that may occur during spray leads to accumulation of massive amount of fipronil in environment in the form of inhalable mist particles. Currently, exposure to the phenylpyrazole pesticides is a global health issue and concerns have been raised as regards relative safety of this pesticides group because of the wide spread use, their toxicity and release into the environment. As human population is getting into continuous long-term contact with most commonly used insecticides in Pakistan and worldwide through inhalation, so it seemed relevant to investigate adverse effects of pesticides inhalation. The present study was carried out to investigate the effects of acute and sub-acute fipronil inhalation on reproduction in male laboratory rats. For carrying out the study, we designed a nose only inhalation chamber designed using fiber glass, restraining bottles and piping. Jet nebulizer was being used for delivery of liquid fipronil in the form of mist particles (inhalable, diameter < 0.5 micron). Adult male laboratory rats were divided into four groups for two different experiments. Controls were given inhalation exposure of distilled water mist for respective duration in each experiment. Acute treatment group received exposure to fipronil for 1 h and 45 min per day (4 days) while sub-acute treatment group received fipronil exposure for 30 min per day (15 days). After respective time period, animals were dissected and blood along with testes and epididymes were collected for analysis. Serum was separated for the determination of ROS, TBARS, SOD and testosterone. Testes were collected for histomorphological analysis, testicular ROS, TBARS

and SOD. Epididymis was processed for sperm count. Paired sample t-test was used for statistical analysis. $P < 0.05$ was considered significant difference. Results demonstrated that acute dose of fipronil mist (1 h 45 min per day for 4 days) led to a significant change in the body weights of animals, no significant change in reproductive organs weights, nonsignificant ($P=0.521$) increase in serum ROS activity, a highly significant ($P < 0.001$) increase in testicular ROS level, no significant change in serum TBARS ($P=0.308$) and testicular TBARS ($P=0.377$) level, significant increase in both serum ($P < 0.001$) and testicular ($P < 0.05$) SOD activity, a highly significant ($P < 0.001$) decrease in serum testosterone concentration and a highly significant ($P < 0.0001$) decrease in epididymal sperm count. Testes of fipronil treated rats showed significant histomorphological changes in the structure such as ruptured epithelium, reduced Leydig cells and widened lumen. Sub-acute dose of fipronil mist (30 min per day for 15 days) led to a significant change in body weights of treated animals, a non-significant change in reproductive organs weights, a significant ($P < 0.05$) increase in serum ROS activity, highly significant ($P < 0.001$) increase in testicular ROS level, no significant change in serum ($P=0.202$) and testicular ($P=0.415$) TBARS level, a significant increase in serum ($P < 0.05$) and testicular ($P < 0.05$) SOD activity, a significant ($P < 0.05$) decrease in serum testosterone concentration and significant ($P < 0.05$) decrease in epididymal sperm count. Testes of rats treated with sub-acute dose of fipronil showed significant decrease in seminiferous tubules, Leydig cells and spermatocytes. The current study concludes that inhalation of fipronil may have an inhibitory effect on the male reproduction suggesting that long-term exposure to fipronil may cause infertility. Decreased testosterone concentration suggests that fipronil via inhalation may target hypothalamic-pituitary-gonadal axis leading to extremely low sperm count. Further investigations required to be carried out to determine the exact mechanism of infertility caused by fipronil inhalation.

**AMELIORATING EFFECT OF VITAMIN C AGAINST BUPROFEZIN-INDUCED
PHYSIOLOGICAL ALTERATIONS, OXIDATIVE STRESS AND GENOTOXIC DAMAGE IN
COMMON
CARP (*CYPRINUS CARPIO* L.)**

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Pesticides are serious health hazards to targeted and non-targeted species due to their diverse use in agricultural fields. Buprofezin (BPFN) is a thiadiazine family insecticide that acts as an insect growth regulator. The present study was undertaken to evaluate buprofezin-induced alterations on growth, survival, behavior, tissue antioxidant enzymes and genotoxicity or the extent of DNA damage in *Cyprinus carpio* and also to explore possible protective role of Vitamin C against such alterations. Presently, fish were divided into ten groups, the control, test and amelioration groups. Fish in the test group was exposed to sub-lethal concentration of buprofezin (BPFN, 100 mg.L⁻¹; 1.072×10^{-6} mol.L⁻¹) for 96 h. In amelioration groups, fish were treated with 25 mg/L and 50 mg/L Vitamin C, each for 1 and 3 weeks. Significance level was $p < 0.001$. Buprofezin exposure caused significant increase in ROS and lipid peroxidation (TBARS) level, while the activities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and non-enzymatic reduced glutathione (GSH) in liver, kidney, brain and gill tissues decreased significantly. Coincident significant decrease in condition factor, hepatosomatic index and survival rate was also observed along with behavioral alterations. Buprofezin application induced genotoxicity in kidney and brain of *Cyprinus carpio*. Vitamin C administration post buprofezin treatment not only increased the growth and survival rate but also cured behavioral abnormalities. Application of Vitamin C appears to have ameliorated toxic effects of buprofezin by decreasing ROS and TBARS, with a concomitant significant increase in the levels of antioxidant enzymes and glutathione in liver, kidney, gills and brain tissues. High dose (50 mg/L) of Vitamin C for 3 weeks reversed the DNA alterations i.e., mean tail length and % tail DNA decreased significantly in brain and kidney tissues on 3 weeks post Vitamin C (50 mg/L) treatment. In summary, results of present study demonstrated that buprofezin exposure induced oxidative stress and genotoxicity in *Cyprinus carpio* and that Vitamin C acts as an ameliorating agent against buprofezin-induced toxicity.

EFFECT OF FIPRONIL ON HEMATOLOGICAL PARAMETERS OF RHESUS MONKEYS**Muhammad Nazeer Khan and Irfan Zia Qureshi****Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 45320 Pakistan*Corresponding Author: irfanzia@qau.edu.pk

With exponential increases in human population, food consumption has increased. To meet increasing demand of food, humans are in constant struggle to increase per capita by using different agrochemicals with different ways to increase the agricultural yield. To evaluate and compare the risk factors and toxicity of one of these chemicals, the fipronil to spray-men, we used rhesus monkeys as suitable models. Monkeys were divided into control and treatment groups each contained three animals. Presently, chair restraint adult rhesus monkeys were exposed to fipronil field spray at the concentration of 0.3 mg.ml⁻¹ of distilled water through inhalation. Nephrotoxic and hepatotoxic effects were analyzed by measuring the liver enzymes, alanine transaminase (ALT), aspartate transaminase (AST) and serum creatinine level. Fipronil treated monkeys showed significantly increased (P-value = 0.000461, 0.0681 and 0.00656 respectively) levels of these enzymes as compared to the control group. Furthermore, significant difference was found in red and white blood cells count (P-value 0.0139 and 0.00642) in fipronil treated monkeys as compared to controls. The present study concludes that fipronil inhalation can cause severe damage to liver and kidney spray-men get passively exposed to fipronil in agricultural fields. Further research is required to explore in detail fipronil toxicity as regards normal physiological functions of animal and human bodies.

ANALYSIS OF ADVERSE EFFECTS OF HEAVY METALS (CADMIUM AND MERCURY) ON LIVER FUNCTIONS AND PROTECTIVE ROLE OF ASCORBIC ACID IN RABBITS**Shumaila Mumtaz¹, Shaukat Ali^{1,*}, Saiqa Andleeb¹ and Hafiz Abdullah Shakir²**¹*Department of Zoology, University of Azad Jammu and Kashmir Muzaffarabad, Pakistan.*²*Department of Zoology, University of the Punjab, Lahore, Pakistan**Corresponding Author: shuakatali134@yahoo.com

Mercury and cadmium are toxic heavy metals that adversely affect some organs in animals and humans. Liver is most sensitive organ to their toxicological effects, due to involvement in elimination of these metals. The aim of this study was to evaluate the toxicological effects of individual and combined exposure to HgCl₂ and CdCl₂ on biochemical parameters of liver, haematological parameters, bioaccumulation in liver and therapeutic role of vitamin C in rabbits against these metals. Rabbits (n=66) were categorized into 11 groups having six replicates each group {Cont, Vit C, Cd, Cd+Vit (Prevention), Cd+Vit (Treatment), Hg, Hg+Vit (P), Hg+Vit (T), Cd+Hg, Cd+Hg+Vit (P), Cd+Hg+Vit (T)}. They were treated with orally administration of HgCl₂ (1.2 mg/kg), CdCl₂ (1.5 mg/kg) and vitamin C (150 mg/kg body weight) for 28 alternative days. Hematological parameters such as (PCV), (Hb), (MCHC) and biochemical parameters of liver comprising: (ALP), (LDH), (ASAT), Bilirubin, (ALAT), total protein and GGT were conducted by blood sampling. Significant decrease (P≤0.01) was detected in hematological parameters such as MCHC, Hb and PCV in intoxicated rabbits with Cd, Hg and their co-administration. Results of biochemical parameters showed that level of ALAT (Cont: 34.4±2.0 IU/L; Cd: 51.0±1.6 IU/L; Hg: 53.8±1.7 IU/L; ALP (Cont: 37.7±2.0 IU/L; Cd: 50.5±1.3 IU/L; Hg: 51.6±1.1 IU/L), Bilirubin (Cont: 1.8±0.1 µmol/l; Cd: 4.8±0.1 µmol/l; Hg: 4.4±0.1 µmol/l), LDH (Cont: 200.3±4.9 IU/L; Cd: 276.0±10.3 IU/L; Hg: 291.7±6.5 IU/L), GGT (Cont: 7.15±0.37 IU/L; Cd: 15.10±0.45 IU/L; Hg: 13.70±0.61 IU/L) and ASAT (Cont: 6.5±0.4 IU/L; Cd: 11.2±0.4 IU/L; Hg: 11.5±0.3 IU/L) were significantly (P≤0.001) increased. While level of total protein was significantly decreased. Accumulation of Hg and Cd (Hg: 27.33±2.33 mg/kg; Cd: 42.33±1.86 mg/kg) in liver was observed. However, chemotreatment with Vitamin C significantly (P≤0.01) decline the toxicological effects of Cd and Hg but not regain the values similar to control group. This study provides awareness about toxicity of Hg and Cd on biochemical and hematological parameters of rabbits and therapeutic role of Vitamin C against these metals.

EFFECT OF DELTAMETHRIN ON SOME BIOCHEMICAL FEATURES IN MARINE CYANOBACTERIA**Saira Bano¹, Zaib-un-Nisa Burhan¹ Munawwer Rasheed^{1,2*}**¹*Center of Excellence in Marine Biology, University of Karachi-75270, Pakistan*²*Department of Chemistry University of Karachi-75270, Pakistan*

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Cyanobacterial strains (MB.1007 and MB.1010) isolated from Karachi coast were treated with different concentrations of deltamethrin. Deltamethrin is widely used pesticide. Deltamethrin has shown deleterious effect on both strain, growth rate decrease with increasing deltamethrin concentration. Biochemical analysis of these strains show significant decrease in total carbohydrates when compared with untreated cultures. However, the total proteins significantly increased, indicating some defense pattern against the lethal effects of deltamethrin.

IN VIVO HEPATOPROTECTIVE EFFECT OF QUERCETIN AGAINST ZnO NANOPARTICLE INDUCED LIVER DAMAGE IN ALBINO MICE**Sumera Sajjad*, Husna Malik, Laiba Saeed and Rabia Anjum***Department of Zoology, Lahore College for Women University, Lahore*

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The present study was conducted to evaluate the hepatoprotective effect of quercetin on liver of mice against subacute toxicity of ZnO nanoparticles. Twenty four healthy adult male albino mice were divided into four groups (n= 6). Group I (Control) received 0.3ml of 0.9% saline solution, Group II received quercetin (100mg/kg b.w), Group III received ZnO nanoparticles (50 mg/kg), and Group IV received quercetin along with ZnO nanoparticles (50 mg/kg) for 21 days. Blood sampling was done after 7th and 21st day of experimental procedure. Body weight and hepatosomatic index was calculated. Liver enzymes (AST, ALT and ALP) were estimated through biochemical kits. Liver tissue was stored in the 30% formalin until it was used by microtomy for the assessment of liver damage. All the data was statistically analyzed by using Dunnett T3 test. ZnO nanoparticles caused significant rise in body weight, liver weight and serum AST, ALT and ALP levels. ZnO nanoparticles displayed infiltration in central vein, congestion of sinusoidal spaces and necrosis in the hepatocytes after 21 days. The quercetin treated group showed normal level of liver enzymes along with normal central vein with few infiltrations; otherwise all the structures are same to that of control group. The animals which received both quercetin and ZnO nanoparticles declined the level of liver enzymes and exhibited infiltration in central vein and necrosis but less than to that of only ZnO nanoparticle given group. Concomitant administration of quercetin along with ZnO-NPs showed protection against ZnO nanoparticles induced liver damage.

COMPARISON OF HISTOPATHOLOGICAL DAMAGE IN TISSUES OF GRASS CARP AFTER PHTHALATE EXPOSURE**Maryam Tariq and Ghazala Jabeen***Department of Zoology, Lahore College for Women University, Lahore.*

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In this study, the healthy juveniles of grass carp were exposed to phthalates, Di methyl phthalate(DMP), Di-n octyl phthalate(DnOP) and their mixture for 3, 6 and 15 days, respectively via water-borne treatment. Behavioral and histo-pathological alterations in gills and cardiac muscle were examined in juveniles of *C. idella*. Analysis of C.F for fish health and meat quality assessment revealed that C.F values of 6- and 15-days phthalate exposures showed significant lower mean values as compared to the 3-days exposed group indicating poor meat quality and toxicity impact of phthalates. Physicochemical analysis of treated fish showed that the

values of pH, TDS, alkalinity and total hardness were within the permissible range referred by WHO and EPA for intensive culture of freshwater fishes while EC values in treated fish were above the permissible limit referred by WHO and EPA. Significant histological alterations in gills were fusion of primary and secondary gill lamellae, epitheliocystis, aneurysm, Haemorrhage, epithelial lifting, necrosis and hyperplasia while histological alterations in heart of exposed fish were atrophy, severe alteration in heart cell arrangement and disintegration of cardiac muscle bundles. HAI (Gills) for phthalate exposed fish followed the order: $HAI_{3\text{-day}} > HAI_{6\text{-day}} > HAI_{15\text{-day}}$. HAI (Heart) for water-borne phthalate exposed fish was in the magnitude of $HAI_{3\text{-day}} > HAI_{15\text{-day}} > HAI_{6\text{-day}}$.

METALLIC NANOPARTICLES CAUSE HAEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS IN *LABEO ROHITA* JUVENILES

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Nanoparticles are being widely used in various fields of biology, for instance, drug delivery, antibacterial applications, nanomedicines and tissue engineering. Therefore, in view of their extensive use it is important to investigate their potential effects on animal systems/tissues. The present study was designed to explore the roles of different metallic nanoparticles on juveniles of *Labeo rohita* (Rohu). The chosen metallic nanoparticles for this study include, Cobalt based nanoparticles (CoNPs), Nickel based nanoparticles (NiNPs), and Zinc oxide nanoparticles (ZnONPs). Effects of nanoparticles on fish survival, growth, behavior, hematology and biochemical parameters were evaluated. Metallic nanoparticles were synthesized by chemical reduction routes. Our results showed that CoNPs caused little or no alteration in fish behavior. However, they caused low leukocyte and erythrocyte counts with erythrocyte having clumped and less defined shape. NiNPs caused disturbed behavior, low leukocyte and erythrocyte count and low total protein levels. Liver histology of nickel treated fish showed more degenerated hepatocytes and necrosis. ZnO NPs were found to be acutely toxic. The behavior of fish was noticed to be disturbed. They retarded the growth and altered the haematological parameters of fish.

TOXICOLOGICAL EFFECT OF ESFENVALERATE ON CARBOHYDRATE METABOLISM OF STORED GRAIN PEST, *TROGODERMA GRANARIUM*

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The aim of present research was to evaluate the toxicity of esfenvalerate in 4th and 6th instar larvae of a stored grain pest *Trogoderma granarium* and to determine the toxic effect of sublethal doses of esfenvalerate on carbohydrate metabolism. The age wise homogenous culture of two populations viz., Lahore and Okara were maintained at 35±2°C and 60±5 relative humidity. The LC 50 values of esfenvalerate for 4th and 6th instar larvae of Lahore population was 34.29 and 28.05ppm respectively, while 39.30 and 32.67ppm were for the 4th and 6th instar larvae of Okara population, respectively. The sub lethal dose of esfenvalerate (LC₂₀) significantly reduced in the carbohydrates metabolites i.e., glycogen, glucose, trehalose with reference to their control (untreated group). Among carbohydrate metabolizing enzymes, the activities of trehalase, amylase and invertase were significantly depleted after treatment with sub-lethal dose of esfenvalerate as compared to control. This piece of work may help to understand the biochemical response of *T. granarium* induced by sub-lethal dose of esfenvalerate suggest that infestation caused by *T. granarium* in godowns could be overcome by calculating lethal dose of esfenvalerate.

LOW DOSE CHRONIC TOXICITY OF BISPHENOL A (BPA) ON THE HEPATIC TRACE METAL LEVELS AND PROTEIN PROFILE OF ADULT MALE WISTAR RATS

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Bisphenol A (BPA) is one of the widely used plasticizer and known endocrine disruptor due to its ability to derail body metabolic functions and adverse effect on the vital organs. The present work outlined the chronic effect of low dose BPA (10mg/kg) on level of hepatic trace metals and protein profile of wistar rats. To conduct the research work, animals were divided into two groups; n=5. Group 1 was given no treatment (control) while as an experimental group 2 was given low dose BPA for 12 weeks. Statistically significant decrease was noted in zinc and copper concentrations while non-significant change was observed for magnesium concentration through atomic absorption spectroscopy (AAS). SDS-PAGE was run for hepatic protein profiling and increased expression of protein was observed in group 2 as compared to control group. It can be concluded from the above results that even low dose of BPA cause negative changes in the liver. Hence it can be suggested that BPA alternative should be use so that public health status can be secured.

CONTEMPLATING TOXICITY OF ATRAZINE ON UREA, CREATININE AND URIC ACID OF FRESH WATER FISH, *CTENOPHARYNGODON IDELLA*, KHYBER PAKHTUNKHWA, PAKISTAN

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Herbicides are utilized in combating herbs and enhancing productivity of agriculture, but they find avenues to get into water bodies, affecting aquatic fauna, particularly fish. The current effort accordingly focused to contemplate toxicity of sub lethal concentration of herbicide i.e. atrazine by estimating the urea, creatinine and uric acid of freshwater grass carp, (*Ctenopharyngodon idella*) for (24, 48, 72, 96, 240, 360 and 600 hrs) under the dose (15, 13, 10, 08, 06, 04 and 02 µl L⁻¹) respectively. Control group concentration of urea, creatinine and uric acid was 4.3, 0.86 and 2.2 (mmolL⁻¹) respectively. Concentration of urea obtained after treatment for 24, 48, 72 and 96hrs (acute toxicity) was 12.6, 10.6, 8.8 and 7 (mmolL⁻¹). Creatinine concentration was 1.6, 1.0, 0.5 and 0.3 (mmolL⁻¹) while uric acid concentration obtained was 4.0, 4.8, 5.6 and 7.2 (mmolL⁻¹) respectively. Similarly, the concentration of urea obtained after treatment for 240, 360 and 600hrs (chronic toxicity) was 14, 10.3 and 9.5, (mmolL⁻¹). Creatinine concentration was 1.4, 0.9 and 0.53 (mmolL⁻¹) while uric acid concentration was 4.0, 4.3 and 5.7 (mmolL⁻¹) respectively. In all component, significant inclined in concentration was observed as; P<0.05, P≤0. 01 and P≤0. 001, specifically in acute toxicity groups as compared to chronic toxicity groups, thus showing undesirable effects of atrazine on aquatic fauna present inside water bodies.

DETRIMENTAL EFFECTS OF HEXAVALENT CHROMIUM ON MORPHOLOGY OF SMALL INDIAN MONGOOSE (*HERPESTES JAVANICUS*) MALE NATURALLY EXPOSED TO CHROMIUM POLLUTED ENVIRONMENT IN DISTRICT KASUR, PAKISTAN

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Chromium is known to cause cellular damage inside the body and alter normal physiological processes in

animals. Since chromium is extremely toxic and oncogenic, its direct discharge from tannery industries into surrounding soil and wastewater streams can lead to serious health problems to humans and wild animals. The current study focused to investigating the clinical and physiological impacts of chromium on small Indian mongoose as model wild species of chromium contaminated environment at tannery areas of District Kasur, Pakistan. Adult male specimens (N = 24) were live trapped from February 2015 to January 2016 for clinical and physiological examination. Control samples were taken from the Potohar plateau. Gross morphological examination of experimental area specimens showed decreased body weight with short body length, reduced body activity, kinky and short tail and less body hair (fur) as well as change in fur color compared to normal control samples. Average body weight (29%), heart (22.35%), kidney (10 %), stomach (25%), small intestine (59.6%), large intestine (11%) and testes (10%) weights of experimental area specimens were found decreased compared to control while, on the other side, average liver (19.14%), pancreas (13.4%) and spleen (18.5%) weights were found increased compared to control. Body ($t = 5.65$, $df = 10$, $p = 0.000$), pancreas ($t = 3.66$, $df = 10$, $p = 0.006$), spleen ($t = 3.77$, $df = 10$, $p = 0.005$), heart ($t = 3.65$, $df = 10$, $p = 0.006$) and large intestine ($t = 2.29$, $df = 10$, $p = 0.05$) weights were significantly different over control samples. Average head to tail length (15.9%), head length, (1.71%), tail (3.6%), body length between head-tail (24.68%), snout (9.1%), forelimb (7.53%) and hind limb (13.5%) length were also found decreased in experimental area specimens compared to control animals. There was significant difference between body length between head-tail ($t = 6.88$, $df = 10$, $p = 0.000$), snout ($t = 2.63$, $df = 10$, $p = 0.03\%$) and hind limb ($t = 4.80$, $df = 10$, $p = 0.001$) over control samples. Similarly, average length of small (28.9%) and large intestines (30.32%) were found decreased. Average length of small intestine was significantly different ($t = 6.11$, $df = 10$, $p = 0.000$) from control samples, while there was a non-significant difference in length of the large intestine. The gonadosomatic index and liver to body weight ratio (1.04 ± 0.32 and 3.76 ± 2.96 , respectively) were significantly ($t = 4.60$, $df = 10$, $p = 0.001$ and $t = 4.53$, $df = 10$, $p = 0.001$, respectively) increased in comparison to control animals (0.83 ± 0.32 and 2.40 ± 4.03 , respectively). Study concludes that Cr being discharged from tanneries into the surrounding environment is up-taken by the small Indian mongoose leading to its morphological alteration.

USE OF FEED ANALYSIS AS A TOOL FOR DETECTION OF HEAVY METAL CONTAMINATION IN BROILER HOUSES

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Intensive agriculture such as poultry is a flourishing industry in Pakistan and helping a lot in the economy of country by providing employment and fulfilling the demand of food for the growing population. Along with this poultry sector is also be considered as one of the major source of emissions like PM, gases, bioaerosols and heavy metal contamination. In the current study feed and litter along with indoor and outdoor air samples were collected from 18 selected controlled environment poultry farms. The samples were digested with 3:1 nitric acid and per chloric acid and later be analyzed for Inductively Coupled Plasma Atomic Mass Spectrometry (ICPA-MS). The various essential metals such as Ca, Cu, Fe, K, Mg, Na, P and Zn were studied along with non-essential metals such as Cd, Cr, Hg, Mn, Ni and Pb for heavy metal analysis of feed, litter, indoor and outdoor samples. The mean metal concentrations was reported in mg/kg for feed and litter samples and in $\mu\text{g}/\text{m}^3$ for indoor and outdoor environments. The maximum mean concentration of heavy metals in different feed, litter, indoor and outdoor samples was found to be 9685 ± 3919 , 2038 ± 3449 , 37.72 ± 15.41 and 39.9 ± 15.8 for Ca; 48.13 ± 31 , 17.81 ± 34 , 0.504 ± 0.56 and 0.37 ± 0.40 for Cu; 340 ± 78 , 153.11 ± 233 , 8.672 ± 4.76 and 4.98 ± 3.07 for Fe; 10686 ± 1646 , 3061.8 ± 4828 , 29.4 ± 8.05 and 20.1 ± 2.84 for K; 2057 ± 222 , 600 ± 392 , 18.88 ± 11.30 and 11.5 ± 2.46 for Mg; 2718 ± 1069 , 705 ± 596 , 332.83 ± 201.71 and 375.17 ± 141.36 for Na; 7552 ± 894 , 1123 ± 1475 , 5.132 ± 2.6 and 1.42 ± 0.78 for P and 204 ± 39.58 , 57.77 ± 83.83 , 0.94 ± 0.60 and 0.281 ± 0.009 for Zn. The maximum mean concentration for non-essential metals for feed, litter, indoor and outdoor samples was like this as 0.82 ± 0.77 , 0.06 ± 0.07 , 0.04 ± 0.01 and 0.04 ± 0.05 for Cd; 11.4 ± 16.0 , 4.88 ± 9.23 , 0.666 ± 0.58 and 4.83 ± 6.69 for Cr; 6.09 ± 5.56 , 3.88 ± 0.45 , 0.19 ± 0.06 and 1.3 ± 1.60 for

Hg; 205 ± 93.9 , 48.20 ± 64 , 0.320 ± 0.15 and 1.05 ± 1.10 for Mn; 2.37 ± 0.53 , 0.97 ± 0.65 , 0.050 ± 0.02 and 0.04 ± 0.02 for Ni; 5.04 ± 0.90 , 1.36 ± 1.08 , 0.318 ± 0.12 and 0.240 ± 0.23 respectively. It was seen that feed was a representative tool that inform heavy metal concentration in litter and indoor air samples because if metals were higher in feed then found to be higher in litter and indoor air samples as well. However, in the outdoor air samples a regular trend for increase and decrease of metal concentrations according to indoor emissions was not observed that showed it has some other sources as well instead of indoor emissions.

DETERMINATION OF HEAVY METAL CONCENTRATION IN CATFISH *RITA RITA* FROM INDUS RIVER NEAR JAMSHORO, SINDH, PAKISTAN

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Present study was conducted to determined heavy metal concentration in catfish *Rita rita* from Indus River near Jamshoro, Sindh, Pakistan. Different metals like iron, zinc, Chromium, copper, manganese and cobalt were analysis from different organs like gills kidney and liver from male and female respectively. The values of iron were found highest ($12.92 \mu\text{g/lit}$) in liver while the lowest value of cobalt ($0.014 \mu\text{g/lit}$) was found in Kidney. Rests of the metals showed no significant variation and were found within the recommended limits of WHO.

EVALUATION OF RENAL TOXICITY INDUCED BY HEAVY METALS (CADMIUM AND MERCURY) AND THE PROTECTIVE ROLE OF ASCORBIC ACID

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Heavy metals are among the most toxic and dangerous environmental pollutants. The toxicants ultimately may enter the human body through food chain. Cadmium and mercury are considered as most toxic heavy metals their intake via environmental sources may cause fatal implications. Vitamin C is an important chain-breaking antioxidant and enzyme co-factor. The main purpose of recent study was to evaluate the toxicological effects of cadmium chloride and mercury chloride on body weights, kidneys, biochemical parameters, bioaccumulation in kidneys and also to elucidate the protective effect of vitamin C in rabbits against these metals. In this study, cadmium chloride, mercury chloride and vitamin C were orally administered to the rabbits at dose rate of 1.5 mg/kg, 1.2 mg/kg and 150 mg/kg of the body weight respectively for group (Cont, Vit C, Cd, Cd+Vit (Prevention), Cd+Vit (Treatment), Hg, Hg+Vit (Prevention), Hg+Vit (Treatment), Cd+Hg, Cd+Hg+Vit (Prevention) Cd+Hg+Vit (Treatment). After the biometric determination of all experimental rabbits biochemical parameters of interest were measured viz. creatinine, cystatin C, uric acid, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured by using the respective kits available commercially. The present study showed that by cadmium chloride, mercury chloride and their co-administration, body weight of all rabbits were reduced significantly in all groups except control and vitamin C treated groups. Biochemical parameters like creatinine (mean \pm SEM $28.3 \pm 1.1 \mu\text{mol/l}$), cystatin C (mean \pm SEM $1860.0 \pm 38.4 \text{ ng/ml}$), uric acid (mean \pm SEM $4.8 \pm 0.1 \text{ mg/d}$), ALP (mean \pm SEM $50.5 \pm 1.3 \text{ IU/L}$) and LDH (mean \pm SEM $291.7 \pm 6.5 \text{ IU/L}$) were significantly ($P < 0.05$) increased due to administration of these metals but chemo-treatment with vitamin C reduces the effects of cadmium chloride and mercury chloride but not regain the values similar to control group. Because of the bio-accumulative nature of cadmium chloride and mercury chloride these metals were absorbed in kidneys of rabbits which lead to deleterious effects on them. This study therefore, provides an insight about the toxicity of the cadmium chloride, mercury chloride and their

combination on biochemical parameters and kidneys of the rabbits and the ameliorating potential of vitamin C against these metals.

PHARMACOLOGICAL INTERVENTION BY VITAMIN C FOR TOXICOLOGICAL EFFECTS INDUCED BY HEAVY METALS (CADMIUM AND MERCURY) ON BLOOD AND THYROID GLAND

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Cadmium and Mercury are non-biodegradable heavy metals and widely spread pollutant and toxicant. Being heavy metals they cause adverse effects via affecting thyroid gland and metabolism along with deleterious blood disorders and immunological effects in rabbits. Current study was aimed to determine the effect of cadmium and mercury on thyroid function, haematological and biochemical toxicity of individual metals and their co-administration, their bioaccumulation in vital organs as well as to evaluate the protective antioxidant role of vitamin C against these metals. For this purpose, 66 rabbits were randomly divided into 11 groups having six replicates each as group: (Cont, Vit C, Cd, Cd+Vit (Prevention), Cd+Vit (Treatment), Hg, Hg+Vit (Prevention), Hg+Vit (Treatment), Cd+Hg, Cd+Hg+Vit (Prevention), Cd+Hg+Vit (Treatment)). Oral permissible dose was 1.5 mg/kg body weight of cadmium chloride, 1.2 mg/kg body weight of mercury chloride and 150 mg/kg body weight of vitamin C for 28 consecutive days. The control group received the same volume of distilled water. Weight of rabbits was closely monitored and whole blood was collected for analysis of biochemical (T3, T4, TSH, Triglycerides, creatinine) and haematological parameters (Hb, MCHC, Total protein). The results of this study showed significant decrease ($p < 0.05$) in hemoglobin concentration, mean corpuscular hemoglobin concentration and hematocrit with high concentrations of mercury and the combination of cadmium and mercury. Analysis of different biochemical parameters at chemo-treatment revealed that in comparison to the rabbits of the control group: 3, 5-triiodothyronine (cont. 0.74 ± 0.07 ng/ml to Cd 0.38 ± 0.02 ng/ml, Hg 0.46 ± 0.03 ng/ml), Thyroxine (cont. 45.5 ± 1.5 ng/ml to Cd 25.8 ± 1.4 ng/ml, Hg 23.2 ± 1.6 ng/ml) and Total protein (cont. 7.0 ± 0.4 g/dl to Cd 4.4 ± 0.1 g/dl, Hg 3.7 ± 0.2 g/dl) were significantly decreased ($p < 0.05$) while Thyroid stimulating hormone (cont. 0.09 ± 0.01 nmol/l to Cd 0.19 ± 0.01 nmol/l, Hg 0.23 ± 0.01 nmol/l), creatinine (cont. 15.5 ± 0.7 μ mol/l to Cd 28.3 ± 1.1 μ mol/l, Hg 24.5 ± 1.0 μ mol/l), and Triglyceride (cont. 1.74 ± 0.08 nmol/l to Cd 4.42 ± 0.18 nmol/l, Hg 3.90 ± 0.10 nmol/l) were significantly ($P < 0.05$) increased but chemo treatment vitamin C reduces the effects of cadmium chloride, mercury chloride and their co-administration but not regain the values similar to control group. This indicates that co-administration of mercury; cadmium and vitamin C had a protective effect on the potential harmful metals. The Cd and Hg also found to accumulate in vital organs when measured by atomic absorption spectrophotometer. This study therefore, concludes that cadmium chloride and mercury chloride are toxic and tend to bioaccumulate in vital organs and their toxic action can be subdued by vitamin C in biological systems.

ASSESSMENT OF TOXICOLOGICAL EFFECTS OF HEAVY METALS ON HEART AND PHARMACOLOGICAL INTERVENTION BY VITAMIN C IN RABBITS

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Heavy metals are environmental toxins. The toxicants ultimately may enter the human body through food chain. Mercury and cadmium are considered as more toxic among heavy metals. Cadmium and mercury intake by rabbits leads to fatal effects. Main objective of this study was to assess the effects of the cadmium chloride and mercury chloride on heart and hematological parameters were estimated and also to measure its

bioaccumulation in heart tissue. Against these metals vitamin C act as anti-oxidative agent. The tolerable dose was 1.5 mg/kg of CdCl₂, 1.2 mg/kg of HgCl₂ and 150 mg/kg of vitamin C administered orally to rabbits of each group {Cont., Vit C, Cd, Cd+Vit (P), Cd+Vit (T), Hg, Hg+Vit (P), Hg+Vit (T), Cd+Hg, Cd+Hg+Vit (P) Cd+Hg+Vit (T)}. After every week weight was measured. Blood collected from each group at various time points after post treatment. Analysis of different hematological (Hb, PCV, MCHC, total protein) and biochemical parameters (LDL, HDL, cholesterol, CK) was done by respective kits. Following results was observed weight of the rabbits were reduced except control and Vitamin C group increased significantly. Highest significant elevation in the weight of Hg+Vit (P) was observed. At the end of exposure, significant decrease ($P < 0.05$) was observed in Hb, MCHC and hematocrit. Results of the biochemical parameters at chemo prevention shown that in comparison to control group Cholesterol (0.7 ± 0.0 mmol/l), Creatin kinase (2985.2 ± 114 IU/L), LDL (20.35 ± 1.31 mg/dl) significantly ($P < 0.05$) increased while HDL (84.78 ± 4.30 mg/dl) was significantly ($P < 0.05$) decreased but chemo treatment vitamin C reduces the effects of cadmium chloride and mercury chloride but not regain the values similar to control group. Bioaccumulation of cadmium and mercury was found to be higher in heart that produce harmful effect on them. This study therefore, provides an awareness about the toxicity of the mercury chloride and cadmium chloride, on biochemical parameters of the rabbits and protective role of vitamin C against these metals.

ROLE OF ZINC IN UPTAKE OF CADMIUM BY WHEAT PLANTS IN GREEN HOUSE

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Heavy metals have become primary pollutant of agriculture which cause chronic health problems in peoples as well in different living organisms. In the present study, role of Zinc (Zn) was assessed in the uptake of Cadmium (Cd) by wheat plants (*Triticum aestivum*) in green house. For this purpose, different concentration of Cd and Zn and combination of both metals was added in the soil. Transfer factor of Cd from soil to plants was higher at low concentrations as compared to high concentrations. However, transfer of Cd from soil to plant decreases significantly in the presence of Zn. Transfer factor of Zn increases with concentration but its transfer decreases in presence of Cd at higher concentrations. The bioaccumulation of Cd in leaves and grain increases with the increases of Cd concentration in soil but opposite trend was recorded for Zn. In leaves and grains exposed to Cd and Zn combination, Cd bioaccumulation decreases in all concentration. The daily metal intake (DMI) and human health risk index (HRI) was higher when wheat plants were grown in the presence of Cd concentrations as compared to Cd and Zn combination. In conclusion, Zn can be used to slow down the transfer rate of cadmium from soil to plants and its bioaccumulation at higher trophic levels.

TOXIC EFFECT OF ABAMECTIN AND EMAMECTIN BENZOATE ON PROTEIN METABOLISM AND DETOXIFICATION ENZYMES IN *TROGODERMA GRANARIUM* (KHAPRA BEETLE)

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Trogodema granarium is highly dangerous, at its larval stage, for stored cereal products as its infestation make them unmarketable due to depletion of important nutrients. Insects have developed resistance due to excessive use of pesticides. So, there is a need to use less harmful pesticides like Abamectin and Emamectin benzoate. In this study these two pesticides were used to check their sub lethal effect on protein metabolism and detoxification enzymes in larvae of this storage insect pest. Total four populations, three tolerant populations from Layyah, DG Khan and Gujranwala and one susceptible population from departmental stock, were collected for study. Two, out of four populations with highest (Layyah) and lowest (susceptible) values of LC₅₀ were selected by Probit analysis at 95% fiducial limit. The fourth instar larvae of selected populations were

treated with LC₂₀ dose of Abamectin and Eamectin benzoate for protein metabolites and detoxification enzymes spectrophotometrically. In susceptible population the soluble proteins (20.07%), free amino acids (28.44%), RNA (44.44%) and glutamate oxaloacetate transaminase GOT(81.81%) were significantly decreased whereas soluble protein (66.01%), cholinesterase (24.80%), glutamate pyruvate transaminase GPT (50%), acid phosphatase AcP (63.88%) and alkaline phosphatase AkP (13.63%) activities were increased significantly after exposure of sub lethal dose (LC₂₀, 179.31ppm) of Eamectin benzoate. However, In Layyah population total soluble protein contents(2.55%) and DNA (16.66%) were significantly increased while other biochemical parameters like total proteins (14.76%), free amino acids (80.55%), RNA (33.3%), Cholinesterase (48.73%), GOT (69.23%), GPT (40%), AkP (74%) and AcP (10.52%) were decreased after exposure of sub lethal dose of (LC₂₀, 401.19ppm) Eamectin benzoate. After treatment of sub lethal dose (LC₂₀, 138.70ppm) of Abamectin in susceptible population, significant increase in activities of total proteins (52.83%), GOT (163.63%), DNA (100%) and AcP (72.22%) were increased whereas soluble proteins (25.19%), free amino acids (45.79%), RNA (44.44%), GPT (100%), cholinesterase (32.44%) and AkP (48.48%) were found to be significantly decreased. Although In Layyah population DNA (66.66%), cholinesterase (6.32%), GOT (130.76%), GPT (80%) and AcP (21.05%) activities were significantly raised but total proteins (30.25%), soluble proteins (7.20%), amino acids (91.84%), RNA (33.3%) and AkP (44.21%) activities decreased after exposure of Abamectin (LC₂₀, 321.02). Although Abamectin has been found most effective than the Eamectin benzoate but both are proved to be capable of controlling the Deltamethrin tolerant populations of *T. granarium*. It can be concluded that Abamectin and Eamectin benzoate at sub lethal concentrations produced significant metabolic alterations in protein metabolism and detoxification enzymes in susceptible and tolerant populations.

PATHO-BIOCHEMICAL HEPATOTOXICITY ON EXPOSURE TO CHLORPYRIFOS INSECTICIDE IN BIRDS (*COLUMBA LIVIA*)

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Ecotoxicological effects of insecticides is gaining attention due to their substantial and indiscriminate use in agriculture and households. The present study was carried out to assess histopathological and biochemical hepatotoxicity of chlorpyrifos (CPF) insecticide in birds. A total of 40 mature and healthy rock pigeons (*Columba livia domestica*) weighing mean 310±10 grams were kept in four equal groups as follow group A birds as control group and group C-D birds as insecticide treatment groups. Three treatment groups were given chlorpyrifos insecticide treated feed (seed & grains) at the doses 1/25th, 1/20th and 1/15th of LD₅₀ (1.3mg, 1.6mg and 2.1 mg/kg/day) for consecutive 8 weeks, whereas group A birds given same quantity of untreated food for same period. Birds in treatment groups showed clinical signs of toxicity; excessive salivation, gasping, lethargy, convulsions, frequent defecation and tremors. Treated birds of group (B-D) showed significant decline (P<0.05) in feed intake and body weights as compared to control group. The feed intake remained significantly lower (P<0.05) in treated birds of group D and highly significant (P<0.01) on day 24 and 36 in group C and D. Significant changes (P<0.05) were recorded in serum levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in birds of group B-D respectively as compared to control. Treated birds showed pale and yellowish coloration of the liver while control group birds showed darkish brown color of the liver. Histological investigations revealed enlarged hepatocytes with broadened sinusoidal and inflammation of hepatocytes in group B and C. While CPF induced major histological changes in the liver of group D birds such as; vacuolation and deterioration of the hepatic cells, hemorrhages and hepatic fibrosis. The results indicated that chlorpyrifos may induce moderate to severe hepatic alterations in birds with proportion to remarkable decline in the population of several avian species.

EVALUATION OF TOXICOLOGICAL EFFECTS OF LEAD ACETATE ON BLOOD OF BROILER CHICKS

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The present study was conducted to estimate the effects of lead acetate that was administered orally at different dose levels in broiler chicks. In the experiment, thirty healthy chicks were selected and then divided into five groups (six chicks in each group). Group A was kept as un-treated or control. Whereas, Groups B, C, D, and E were treated with lead acetate once in a day at a rate of 71, 142, 213 and 284 mg/kg of body weight respectively for twenty five days consecutively. Different biochemical parameters of lungs including; glutamate pyruvate transaminase (GTP), alkaline phosphatase (ALP), aspartate amino transferase (ASAT), lactate dehydrogenase (LDH), superoxide dismutase (SOD), HDL, LDL, ALAT and glutathione levels were evaluated spectrophotometrically. A significant ($P<0.05$) increase was recorded in GPT, ALP, ASAT, ALAT and LDH levels in all the treated groups. While, SOD and glutathione levels were found to be decreased in treated groups. The GPT, ALP, ASAT, and LDH levels were observed to be significantly ($P<0.05$) higher in groups treated with high doses of 213 and 284 mg/kg of body weight of lead acetate. Variance analysis showed that the DATA were significant not only from the single factor (dose/days) point of view, but also from their combined effect (dose rate \times different days of analysis), which gave significant results with a P value less than 0.05. The mortality rate was observed as 0% for all the groups. Postmortem analysis showed gross pathological changes on liver, lungs, kidneys, intestines, gall bladder and heart at high dose levels of lead acetate. The lead acetate was also found to be accumulated in lungs ($39.8 \pm 3.0 \mu\text{g/g}$). These results showed that lead possessed significant capability of bioaccumulation. However, it also revealed that lead toxicity increased as the dose rate increased and higher doses of lead caused lungs toxicity in broiler chickens.

TOXICOLOGICAL EFFECTS OF COPPER OXIDE ON *CYPRINUS CARPIO* AND ITS MITIGATION BY *MORINGA OLEIFERA* SEEDS EXTRACT

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Copper oxide is widely used as a pesticide, generally at higher concentrations. Unlike other organic pesticides, copper does not degrade, but rather enters a complex biogeochemical cycle, specifically in aquatic systems. Emerging evidence shows that very low, sublethal copper levels can adversely affect the sense of smell and behavior of fish. Therefore, present study was planned to investigate the toxic effects of copper oxide in freshwater fish *Cyprinus carpio* and mitigated its toxicity by the seed extracts of *Moringa oleifera*. For *in vivo* assessment of protective effect of *Moringa oleifera* seeds extract $n=120$ *C. carpio* of (40-45g) were randomly allocated into 12 experimental tanks (10 fish/aquaria of capacity 40L) 24 h prior to the experiment. The experimental fish were administered to water-born exposure of different dose levels (100 or 200 or 300 mg/l) of *Moringa oleifera* seed extract along with the 1.5 mg/l of copper oxide for 28 days. The *Moringa oleifera* seeds extract showed significant ameliorative role on the antioxidant defense in response to elevated levels of copper oxide induced oxidative stress and haematological parameters. It also played a protective role by the suppression of histological alterations in gills and liver and kidney of fish exposed to copper oxide. Therefore, it is concluded that copper oxide induced toxicity in the *Cyprinus carpio* and *Moringa oleifera* seeds extract exhibits protective effect against the toxicity of copper oxide.

BEHAVIORAL RESPONSES OF *LABEO ROHITA* UNDER THE ACUTE EXPOSURE OF CHLORPYRIFOS

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The present research work was designed to determine the 96-hr LC₅₀ and lethal concentration of chlorpyrifos for *L. rohita*. The mean 96-hr LC₅₀ and lethal concentration of chlorpyrifos for *L. rohita* was estimated as 10.39±0.03 and 15.31±0.05 µg/L⁻¹, respectively. Behavioral changes of *Labeo rohita* were also observed. Exposed fish showed rapid opercular movement, profuse mucus secretions and imbalance swimming, increased surface activity, loss of equilibrium, convulsion, body discoloration, and decreased hyperactivity and fin movement before the death were observed fish tried to jump out of exposed medium, which proves the avoidance behavior of fish against pesticide toxicity.

EVALUATION OF GENOTOXIC POTENTIAL OF PHTHALATE ON GRASS CARP (*CTENOPHARYNGODON IDELLA*)

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Evaluation of genotoxic potential of phthalates in terms of micronucleus assay on *Ctenopharyngodon idella* was carried out. Fish was exposed to dimethyl phthalate (DMP), di-n-octyl phthalate (D-n-OP) and their mixture under sub-acute, acute and chronic toxicity at different concentrations (1.5µg/L⁻¹, 4µg/L⁻¹, and 10µg/L⁻¹) for 3, 6 and 15 days, respectively. Changes in total serum protein level were analyzed with all concentrations and exposure periods and a significantly higher decrease of total serum protein of exposed fish was observed in the treatment groups as compared to control. Results indicated genotoxic and mutagenic damage in erythrocytes of *C. idella* demonstrating nine major nuclear abnormalities (NA) other than micronuclei (MN). Among the examined abnormalities, notched nuclei were the most frequent while nucleoplasmic bridged RBCs showed the lowest frequency in all groups. Frequencies increased significantly at (p<0.001) in concentration and time dependent manner. Hematological parameters, RBCs, Hgb, Hct, MCHC and platelet count showed significantly higher values after exposure to mixture of phthalates (DMP+D-n-OP) at P<0.001. Highly elevated values of hematological parameters at higher exposures indicated an increased level in RBCs production to counter balance and minimize the toxic impact on fish immune system survival rates.

AN APHRODISIAC ETHANOLIC SEED EXTRACT OF *CHENOPODIUM ALBUM* L. AND VITAMIN C AS EFFECTIVE THERAPEUTIC AGENTS AGAINST MERCURY INDUCED TESTICULAR TOXICITY IN SPRAGUE-DAWLEY RATS: A COMPARATIVE HISTOPATHOLOGICAL APPROACH

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Mercuric Chloride (HgCl₂) is notorious for its toxic effects and is naturally found in all biotic factors occurring in all physical states and is readily available in the atmosphere. HgCl₂ is used in chlor-alkali industry, dental amalgams, photography and as pesticide. Mostly human are prone to low doses of Mercury by occupational exposures and through consumption of large amount of sea food. The study was designed to

investigate the comparative curative properties of *Chenopodium album* and Vitamin C against HgCl₂ induced testicular toxicity. Twenty adult male Sprague Dawley rats (200-270g) were divided into four groups. Group I was considered as control group being given 1 ml of physiological saline. Group II rats were intoxicated with intra-peritoneal (i.p) dose of HgCl₂ (0.15mg/rat) dissolved in distilled water. Group III was provided with HgCl₂ (0.15mg/rat) i.p alongwith Vitamin C (200mg/kg) given orally. Group IV received HgCl₂ (0.15mg/rat) i.p with ethanolic seed extract of *C. album* (200mg/kg) orally. All the treatments were carried out for 30 days. The rats were dissected and their testes and epididymal tissues were stored for histology while the blood plasma was stored for biochemical and hormonal analysis. Results of these studies showed adverse histopathological changes in testicular tissues due to HgCl₂ induced toxicity. The morphological degenerative changes include highly significant ($p < 0.001$) decrease in percentage area of seminiferous tubules and significant increase in percentage area of lumen and interstitium. It also caused significant increase ($p < 0.01$) in blood urea nitrogen (BUN) levels. Similarly significant results were also obtained for lipid profile. However, these adverse effects were improved by Vitamin C and *C. album* administration, resulting in reversal of histological damage and spermatozoa number. Similarly, BUN levels were reversed in *C. album* co-treated group. Lipid profile values were significantly increased in co-treated groups signifying the increased levels of steroids. Thus the present study concluded that the toxic effects of HgCl₂ can be ameliorated by *C. album* which signifies its therapeutic potential for treatment of male fertility disorders.

EFFECTS OF DIETARY PESTICIDE ON DNA DAMAGE IN PERIPHERAL BLOOD ERYTHROCYTES OF *CIRRHINA MRIGALA*

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Agricultural and industrial effluents contain different toxicants. Among different toxicants, pesticides are now contaminating our aquatic ecosystems in a very drastic manner. Because pesticides are biologically non-degradable so they can cause toxicity in animals through oxidative damage to membrane lipids, DNA and proteins. These pesticides can enter in animal body through water or food/diet. This study reveals that dietary endosulfan can induce genotoxic damage in peripheral blood erythrocytes of 150-day old fish, *Cirrhina mrigala*. Fish were exposed to four different sub-lethal concentrations viz. 10%, 20%, 33% and 50% LD₅₀ of dietary endosulfan, for 30 days along with positive (Cyclophosphamide) and negative control groups, separately. After 30 day exposure period, peripheral blood of chronically exposed fish was examined for percentage of damaged nuclei (%), genetic damage index (GDI) and cumulative tail lengths of comets (μm) by using Comet assay technique. Chronic exposure of dietary endosulfan to *Cirrhina mrigala* induced higher DNA damage in peripheral erythrocytes of fish that varied significantly ($p < 0.05$) with exposure concentrations and control groups. However, the 50% LD₅₀ exposure of dietary endosulfan caused significantly higher percentage of DNA damage, GDI and cumulative tail length to the comets while it remained significantly lower due to negative control treatment. This study also reveals that Comet assay can be used as useful tool for the determination of genotoxic effects of pesticides on fish.

IMPACT OF GREEN TEA EXTRACT ON ACRYLAMIDE INDUCED TOXICITY IN MICE: BIOCHEMICAL AND HISTOPATHOLOGICAL APPROACH

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As a result of heat induced reaction, Acrylamide (ACR) is formed in carbs containing food above 120°C. Current study emphases the antioxidant power of green tea extract (G.T) against the renal and hepatic toxicity

of ACR. Experimental study was comprised of eight groups of albino male mice treated for thirty days. First was control. Second, third and fourth groups were exposed with different concentrations of ACR. Fifth, sixth and seventh were co-administered with G.T and different doses of ACR while eighth one was treated with G.T only. Mice were dissected on 31st day. Blood samples were collected through cardiac puncture under anesthesia. Liver and kidneys were dissected out for histology. The observations indicated that ACR administration reduced body weight while increased liver and kidney weights. As well as ACR disturbed biochemical parameters like ALT, bilirubin, urea and creatinine levels increased whereas ALP level decreased. Histopathological examination showed various anomalies like vacuolations, necrosis, hemorrhages, pyknotic nuclei, hypertrophy, sinusoidal dilations, cytoplasmic degenerations, glomerulonephritis, glomerulosclerosis, tubular dilations, tubular hyperplasia, epithelium degeneration, swelling and cellular rupture. However, co-administration of Acrylamide and G.T significantly reduced the multiple toxicities. Therefore, it is concluded that make sure the consumption of G.T and avoid overcooked carbohydrate rich food.

TRAMADOL TOXICITY INDUCED IN MALE MICE *MUS MUSCULUS*

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Tramadol hydrochloride is oral analgesic drug used in the treatment of mild to severe, cancer and non-cancerous chronic pain. It is widely marketed and prescribed in over 90 countries. Its analgesic action results from both norepinephrine and serotonin reuptake inhibition and direct-receptor activation. Present study was conducted on albino mice *Mus musculus* to evaluate its toxic effects on kidney. Animals were divided into four groups, three experimental and one was control group. For the experimental groups, the desired doses (1.75, 3.5, 7.0 µg/g B.W.) were given daily by gavage for 30 days. The mice were weighed weekly. On 31th days, the blood of all groups was collected through intracardial puncture for biochemical analysis. Kidneys were dissected out and fixed in Bouin's fixative for the histological analysis. Biochemical tests results showed the significant decrease in testosterone level, ALT, AST, alkaline phosphate and urea level by the difference of $p < 0.05$ but creatinine level increased in experimental groups as compared to control group. Morphometric results indicated significant ($p < 0.05$) decrease in body and organs weight. Histopathological observations regarding to the renal tissues showed glomerular sclerosis, wide Bowman's capsule and tubular degeneration. Hence, it is concluded that tramadol has a potential to cause toxicity in mice.

EFFECT OF ELEVATED LEVEL OF CARBON DIOXIDE AND UV RADIATION ON COGNITIVE ABILITY, HEALTH STATUS AND GENERAL MORPHOLOGY OF SURFACE FEEDER CARPS UNDER LABORATORY CONDITION

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The present study was design with the aim to evaluate the effect of global warming on surface feeder carps, *Hypophthalmichthys molitrix* and *Catla catla*. A completely randomize experiment in replicate of three was designed and fingerlings of both species were exposed to elevated carbon dioxide (7.5 mg/L), temperature (28° C) and ultraviolet radiation (UVB/UVA) a condition mimic to global warming for 5 days. Exposure changed the water quality parameter especially DO level and pH and showed significant effect on health, general morphology, body coloration and behavior of both species. The body color of both species become light and their skin appeared as susceptible to parasitic attack. Shoaling behavior of both species was also disturbed, however, *H. molitrix* showed more struggling behavior and had taken more time to capture from home aquaria as compared to *C. catla*. Conversely, *C. catla* showed more pronounced altered life skill

activities (exploratory and anti-predatory behaviors), boldness/activity, olfaction and stimulus oriented response as compared to *H. molitrix*. The results of present study indicated the negative impact of global warming on behavior, health and general morphology of fish.

TISSUE ANALYSIS OF BLACK KITE, HOUSE CROW, HOUSE SPARROW AS AN ENVIRONMENTAL POLLUTION INDICATOR OF HEAVY METALS

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River Ravi, Lahore stretch experienced extensive industrial growth with intense anthropogenic activities. Trace elements i.e., Zn, Cd, Cr, Cu, Pb and Ni were analysed in kidney, muscle and liver tissues of House sparrow, House crow and Black kite for pollution assessment. Zn (ug/g) and Ni (ug/g) were representing highest and lowest concentrations in all biological samples of analysed bird species. The Black kite represented elevated levels of metal accumulations in comparison to House crow and House sparrow. The concentrations recorded in House sparrow were least observed than both other species and that could be explained with respect to their diet and physiological differences i.e., variations in metabolic rates. Metal accumulation levels recorded in all specimens were not seem to cross any threshold limits. The current study recommended further analysis to determine accumulation potential of analysed metals in feathers and blood of these bird individuals, aiming to better understand both the degree and type of exposure to these metals.

DETERMINATION OF HEAVY METALS CONCENTRATION IN THE TISSUES OF SCHIZOTHORAX PLAGIOSTOMUS (HECKEL, 1838) IN RIVER SWAT

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The current study was conducted in river swat at selected sites such as Charbagh, Odigram and Landakai of district Swat. The study was aimed to analyze the bioaccumulation pattern in the tissues such as muscles and gills of freshwater fish *Schizothorax plagiostomus* at the selected sites. *Schizothorax plagiostomus* is economical fresh water belong to class actinopterygii, also known as snow trout. The fish is selected for study because of their nutritive, commercial and consumption values. The samples were collected in triplicate form and the extracted tissues were dissolved by the use of per- chloric and nitric acid along with hotplate for the further dissolution of tissues. The heavy metals like Zinc, lead, chromium and Nickel were determined using Perkin Elmer 2380 atomic absorption spectrophotometer. A great variation occurred in the metals content related to tissue type and sampling sites. High concentration of bioaccumulation was reported at Charbagh site and least occurred at Odigram site. The order of accumulation were Charbagh>Landakai>Odigram. In the same way Cr was the most accumulated heavy metal followed by lead, nickel and Zinc. The order of accumulation were Cr>Pb>Ni>Zn. The concentration was taken in mean \pm standard error. Similarly the order of accumulation of metals at Odigram, Charbagh and Landakai were Cr>Ni>Zn>Pb, Cr>Pb>Ni>Zn and Cr>Pb>Ni>Zn respectively and the mean concentration of the heavy metals such as Zn, Pb, Cr and Ni in the muscles at Odigram site were 0.55, 0.21, 1.60 and 0.92ppm respectively and that in gills were 0.42, 0.60, 1.43 and 0.45ppm respectively. Whereas concentration in the muscle at Charbagh site was 0.13, 1.94, 2.18 and 0.51ppm and in gill was 0.25, 0.04, 2.98 and 0.95ppm respectively. Similarly the mean concentrations in the muscle at Landakai site was 0.08, 0.90, 1.32 and 1.71ppm and that in gill was 0.23, 1.50, 1.76 and 0.94ppm respectively. Data demonstrated that heavy metal levels in the tissues of *Schizothorax plagiostomus* were within permissible limits provided by WHO and thus suitable for human consumption but it could be a matter of concern in the near future due to too much industrialization and other activities.

EFFECT OF DELTAMETHRIN AND EMAMECTIN BENZOATE ON THE WEB BUILDING BEHAVIOR OF AN ORB-WEB SPIDER

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Orb-web spiders, *Neoscona theisi*, were collected from wheat fields of Sargodha and kept in boxes (2 × 2 ft) specially designed for them to observe web building. Spiders were divided into two groups i.e. control and experimental. Control group spiders were treated only with distilled water while in experimental group spiders were exposed to field, intermediate and half field rate concentrations of Deltamethrin and Emamectin benzoate. Observations were made after every 24, 48 and 72 hours of exposure. The pictures of webs were taken with digital camera using black background. The web parameters like number of spirals, radii, diameter, radius, mesh height, capture area and anchoring thread length were computed and compared between two groups. Overall results showed that both insecticides influenced normal web building activity of spiders. Deltamethrin caused more mortality (50%) as compared to Emamectin benzoate (20%) at field rate concentrations. Both insecticides severely affected web building at field and intermediate field rate concentrations while no significant difference from control was observed at half field rate concentrations for both insecticides. Emamectin benzoate though appeared less lethal as compared to Deltamethrin but it showed more sub-lethal effects and affected web building more significantly as compared to Deltamethrin.

STUDIES ON INDUCTION OF NUCLEAR ABNORMALITIES IN PERIPHERAL BLOOD ERYTHROCYTES OF LEAD EXPOSED CATLA CATLA

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Heavy metals are increasing due to anthropogenic activity in the aquatic environment. These metals enter into food chain and cause genetic damage to aquatic fauna. During present study, acute toxic effect of lead was determined in *Catla catla* under controlled pH, total hardness and temperature of water by using static bioassay method. Micronucleus test was used to detect the genotoxic effect of lead on fish in the laboratory. The acute toxicity in terms of 96-hour LC₅₀ and lethal concentration (96-hr exposure period) of lead was calculated as 145.92±0.02, 295.97±0.01mgL⁻¹, respectively for *Catla catla* by using probit analysis method. After acute trial, fish was exposed to two sub lethal concentrations of lead (PbT1 and PbT2), separately. After chronic exposure of lead the blood samples were taken from the caudal vein of fish and processed for investigation of morphological nuclear anomalies (%) i.e. lobbed, blebbed, dumbbell, deshape and binuclei in the peripheral blood cells of *Catla catla* in dose dependent manner. Results revealed concentration dependent increase in the frequency of micronuclei and other nuclear abnormalities.

CHANGES IN SERUM BIOCHEMISTRY OF FISH, CATLA CATLA IN RESPONSE TO METAL TOXICITY

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In the present study an attempt has been made to evaluate the effect of heavy metals i.e. nickel (Ni) and zinc (Zn) on the serum biochemical parameters of *Catla catla*. The fish were exposed to lethal concentrations (96-hr LC₅₀) of selected metals. The studied serum biochemical parameters were sodium (Na), potassium (K),

chloride (Cl), albumin (Alb), urea (U), glucose (Glu), aspartate aminotransferase (Ast) and alanine aminotransferase (Alt). Results revealed a decrease in Na, Cl and Alb levels of metal exposed fish at all exposure periods as compared to control. However, an increase in K, U, Glu, Ast and Alt levels was observed in the metal exposed fish. All the biochemical parameters showed significant dependence on exposure durations in both treated fish except Na and Alb in Zn treated fish. This study indicated that the alterations in serum biochemical parameters may be the result of the target tissue damage and dysfunction induced by the metal and that these parameters can thus be used to assess the toxic effects of metals on organisms.

IMPACT OF THREE SUB-LETHAL DOSES OF THE RODENTICIDES ON HEMATOLOGIC AND HISTOPATHOLOGIC PARAMETERS IN FOUR RODENT SPECIES

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Many rodenticides are used for rodent management. In the present studies two acute rodenticides (zinc phosphide, cholecalciferol) and two anticoagulants (barium carbonate and zinc phosphate) were used on the four-rodent species (*Mus musculus*, *Rattus rattus*, *Bandicota bengalensis* and *Nesokia indica*) to evaluate hematological (blood, serum) and histopathological changes in vital organs (Brain, kidney, liver, lungs and heart) at 1st, 3rd and 5th days of a week in the form of sub-lethal concentration of (0.025, 0.05, 0.075 mg/100 gm bait). Rodents were captured live from the field crops of two different sites (Jhang and Faisalabad) in Central Punjab, Pakistan. The group of each rodent species (n=12) of average weights (with three replications) were selected for these concentrations. Bait consumption in control (plain bait 96:2:2, Wheat: sugar: vegetable oil) and treated group (Plain bait mixed with sub-lethal concentrations) was calculated on daily basis, average reduced body weights and mortality was calculated on alternate days. High mortality ratio was observed with acute group at 0.075mg/100gm bait concentration. Overall body weight was decreased and individual organ weight was statistically increased with acute group compared with control. White blood cells were statistically increased with acute and anticoagulants comparatively with decreased ratios of all other blood parameters in control group. Cholesterol and urea level was significantly ($P<0.05$) increased and serum bilirubin, alkaline phosphatase, serum creatinine was decreased due to acute rodenticides. Significant histopathological changes were observed in liver, lungs and kidney tissues among all rodent species with the oral toxicity of zinc phosphide and cholecalciferol as compared with anticoagulants. It is conceivable from present data that orally given minimum concentrations of acute and anticoagulant rodenticides on alternate days might be useful for rodent management outdoor and provide safety to the non-targeted species.

EFFECT OF CHRONIC EXPOSURE OF METALS ON THE GROWTH PERFORMANCE OF *CHANNA MARULIUS* AND *WALLAGO ATTU*

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To check the growth performance under chronic (water borne) exposure the fish, *Channa marulius* and *Wallago attu* were exposed to sub-lethal i.e 1/3 of LC₅₀ concentrations of chromium and cadmium for 8 weeks. In both treatments, statistically significantly variable responses were determined wet weight gain, feed intake, feed conversion ratio and specific growth rate. The fish reared in control (unstressed) medium gained significantly higher wet weights followed by chromium and cadmium test mediums. The feed intake by *Channa marulius* was significantly maximum as (12.83±0.07 g) in chromium followed by cadmium (12.14±0.05 g) and

control (9.57 ± 0.364) medium. However, feed intake by *Wallago attu* was maximum in control (15.30 ± 0.40) medium followed by chromium (13.08 ± 0.06) and cadmium (11.98 ± 0.03) exposed mediums. The overall cumulative feed intake by *Wallago attu* was significantly higher than *Channa marulius* in all the test mediums. The feed conversion ratio by *Channa marulius* and *Wallago attu* in unstressed test medium was significantly better than fish reared in chromium and cadmium exposed test mediums. The specific growth rate of 20.50 ± 0.52 and 21.78 ± 0.44 was noted for *Channa marulius* and *Wallago attu* in the control medium that was significantly better than chromium and cadmium exposed mediums that showed SGR of (13.62 ± 0.09 and $14.02 \pm$) and (13.55 ± 0.05 and 13.82 ± 0.04).

ANTIDIABETIC EFFECT OF *ALIUM SATIVUM*, *AZADIRACHTA INDICA* AND *TRIGONELLA FOENUM* IN ALLAXON INDUCE DIABETIC RABBITS

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The major objectives of the present experimentations were to determine the effect of bromoxynil on blood, liver and kidney enzyme activities in birds/Japanese quail. For this study, 60 Japanese quails (45 days old) were taken and divided into five groups i.e., 1 control and 4 treatment groups with 12 birds in each group. Bromoxynil was administered to quails at the rate of 1mg/kg body weight/day with one drop of pure water. Blood Parameters that were tested include total RBCs count, hemoglobin concentration, packed cell volume (PCV) mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular value (MCV). RBCs count, PCV and hemoglobin concentration were decreased at significant level with increasing dose. MCV was found significantly decreasing with increasing the dose. MCH was first significantly increased then decreased. MCHC was significantly increased. Other parameters that were tested include creatinine, Aspartate aminotransferase (AST) and urea. These parameters showed a significant increase with increasing the dose. Thus, it was concluded that bromoxynil served as a disturbing substance for liver, kidney and hematological factors tested in the present study.

HEMATOLOGICAL VARIATIONS IN WORKERS OCCUPATIONALLY EXPOSED TO HAZARDOUS CHEMICALS AT PETROL FILLING STATIONS AND AUTOMOBILE WORKSHOPS

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Petroleum products are known to emit pollutants that have a negative impact on environmental and human health. Considering the increased occupational exposure to such products the current study was carried out to investigate hematological changes in petrol pump attendants (PPA) and automobile mechanics (AMM). For this purpose, 29 AMM and 19 PPA were taken as exposed workers and compared with 22 healthy controls. Among hematological parameters, RBCs, Hb and MCH decreased non-significantly in both categories of exposed workers when matched with the controls. Hematocrit showed a significant decline ($p < 0.001$) in PPA as compared with controls however, it varied non-significantly in AMM. MCHC significantly increased ($p < 0.01$) in PPA whereas, varied non-significantly in AMM compared to controls. MCV significantly declined ($p < 0.001$) in PPA and ($p < 0.05$) in AMM as compared with controls. Significant elevations were displayed for WBCs count ($p < 0.001$) in PPA and ($p < 0.01$) in AMM, lymphocytes ($p < 0.05$) in both, monocytes ($p < 0.001$) in PPA, granulocytes ($p < 0.001$) in PPA and ($p < 0.05$) in AMM, neutrophils ($p < 0.01$) in PPA and eosinophil ($p < 0.05$) in PPA when compared to the healthy controls. The PLT count decreased, however, MPV increased non-significantly in both exposed groups when matched to the controls. The decreasing trend in RBCs, Hb, MCH and HCT is correlated with possible risks of anemia and bone marrow suppression. Elevation of body

defense cells has connection with the immune system activation, to combat with the foreign antigens in this case petrol fumes.

POLYAROMATIC HYDROCARBONS (PAHS) RESIDUES IN SEAFOOD ASSOCIATED TO ETIOLOGY OF CANCER AMONG COASTAL VILLAGERS

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This study aims to evaluate the carcinogenic risk of PAHs in the edible fishes and study their associations with cancer incidence among coastal villagers. The carcinogenicity of certain PAHs is well established and contamination of PAH in edible fishes is one of the source of health hazard. A number of fish samples from different coastal villages were collected during last 2016-2017 were analyzed to determine level of 16 PAH residues. The carcinogenic risks of the PAHs were calculated for each village using a health risk assessment approach. Results showed that PAHs concentrations in 25% of the fish samples were higher than the permissible limits. However, no significant difference in fish PAHs concentrations was observed between the risk and control groups ($P > 0.05$), and no correlation was found between PAHs concentrations in fish and cancer incidence in these villages. PAHs were present in the fish tissue of the studied villages, but their carcinogenic risks remained within acceptable limits. PAHs in local fish tissue might not be the major environmental cause of the cancer incidences.

EXTRAHEPATIC HISTOLOGICAL ALTERATIONS WITH LOW DOSE MONOSODIUM GLUTAMATE(MSG) IN ALBINO MICE

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Monosodium glutamate (MSG) - a sodium salt of glutamic acid - known as fifth taste (umami), can produce a unique taste, that improve the quality of food intake by stimulating chemosensory perception and proposed to enhance the taste in various types of patients with cancer, radiation therapy and organ transplantation. The present study was aimed and performed to study deleterious effects of MSG on the extra hepatic organs in male albino mice (*Mus musculus*) during acute phase response up to 24 hours. For the purpose, commercially available reported low dose of MSG was injected intraperitoneally (Ip) in mice. The mice were divided into five groups (C, 3, 6, 12 and 24; n=4) on the basis of time period after intraperitoneal administration of MSG. Control group injected with pyrogen-free normal saline parallel to the experimental groups. Tissue samples were taken after dissection and processed for histological studies. A significant time dependent changes in the histopathology of kidney, spleen and intestine was noted. In treated animals, degeneration of epithelia of renal tubules and dilation of glomeruli was evident. Regarding spleen cellular disruption and degeneration of the white pulp was seen in all tissue sections. The histological finding of intestinal sections showed disorganized architecture of the epithelium and microvilli among all experimental groups. It is inferred from these findings that taste enhancers like MSG must not be used routinely as causes noticeable histological changes. The only way to prevent such changes is to avoid foods containing MSG.

SEABIRDS AS BIOINDICATORS OF PESTICIDE POLLUTION IN MARINE ENVIRONMENT OF KARACHI COAST

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This study aims to determine the biochemical and physiological consequences of marine pollutants on seabirds from Karachi Coast. Seabirds are useful as bioindicators of pesticide pollution in marine environments because they are often at the top of the food chain, ubiquitous, and many are abundant and common, making collecting possible. Seabirds have the advantage of being large, wide-ranging, conspicuous, abundant, long-lived, easily observed, and important to people. Many species are at the top of the food chain where they bioaccumulate contaminants with age. One disadvantage is that many species are migratory, making it difficult to determine where exposure occurred. This can be eliminated by using young or baby birds that obtain all their food from parents. Further, noninvasive collection of feathers can be used to assess pesticide levels, from collected specimen. Marine birds can be used as bioindicators in many ways, including tissue levels of pollutants, epidemiological field studies of effects, and experimental and laboratory studies of dose and effects.

BIOFORTIFICATION OF LOW GRADE CEREALS BY USING SOLID STATE FERMENTATION

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This research work was conducted to apply biofortification technique for enhancement of nutrient contents in fermented foods through solid state fermentation by using *Rhizopus oligosporus*. The aim of the research work was to determine the composition of fermented and nonfermented cereals such as wheat, maize and barley. Different parameters of fermentation such as soaking, boiling, incubation temperature, incubation time, salt concentration and acetic acid concentration were used for the growth of *R. oligosporus* in cereals cake. All the cereals were aerobically incubated with *R. oligosporus* at 35°C for 48 hrs to obtain cakes of cereals. The proximate analysis of fermented and nonfermented cereals was conducted. Moisture contents, ash contents and amount of total proteins were increased in fermented cereals. Similarly dietary fibers have significant importance in human food. The study showed that the amount of these fibers significantly increased after fermentation. Reducing sugars were increased in fermented cereals while amount of total sugars decreased in fermented wheat, maize and barley. Further, activities of amylase, protease and phytase enzymes in wheat, maize and barley before and after fermentation were investigated. The results showed that the activity of amylase, protease and phytase enzymes increased in fermented cereals. In the present work, it was showed that biofortification of cereal grains enhanced the nutrient value of cereals.

STUDY OF HPLC CHARACTERIZED COMPOUNDS AND BIOLOGICAL ACTIVITY UNDER LEAD INDUCED STRESS IN ZEA MAYS L

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Maize is an important food crop with significant contribution in human nutrition. It is successfully grown under diverse soils and climatic conditions throughout the world. Lead is most common heavy metal contaminant in environment. It is not essential element for plants, but plants absorb lead from contaminated soil which lead to hormonal imbalance and induce over production of reactive oxygen in plant species. The current study was designed to evaluate the effects of Pb(NO₃)₂ induced stress on biological activity and bioactive compounds in maize. The plants were subjected under two different concentrations (T1- 0.35 mg/ml and T2-

0.45 mg/ml). Phytochemical screening revealed the presence of alkaloids, coumarins, saponins, tannins and terpenoids in maize. TPC% of plants under lead stress extract was observed more (T1- 45%, T2- 58.42%) as compared to control (36.29%). The cytotoxicity of maize extract was checked using the hemolytic activity against human red blood cells and observed that lead reduced the cytotoxicity as compared to the control. The antioxidant activity of maize extracts induced with lead stress was determined using DPPH method. The scavenging rate was highest (T1- 33.5%, T2- 52%) as compared to control (18.6%). The findings of the research exhibited that the maize extract contains antimicrobial property against selected bacterial and fungal strains. *Escherichia coli* had the maximum activity towards control (25 ± 3.46 mm) while it showed less activity in case of lead stress treatments (T1- 17 ± 1.633 mm, T2- 20 ± 4.08 mm). Zone of inhibition of *Aspergillus niger* was highest amongst other two fungal strains. The HPLC results showed that maize has some phyto-ingredients which may be accountable for cell reinforcement and anti-microbial activity. The extract of maize was analyzed for the biochemical profile like superoxide dismutase, peroxidase, catalase, amylase and protease. Lead stress altered all the activities as compared to control but plants have internal system of regulation to modify these responses. In conclusion, *Z. mays* can be used as an indicator species for lead and the various antioxidants to play a key role in the detoxification of lead induced toxic effects.

MOLECULAR ANALYSIS, CLONING AND TRANSFORMATION OF CP1105 GENE ISOLATED FROM CALOTROPIS PROCERA

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Calotropis procera is a tropical plant that belongs to the family Asclepiadaceae and has been widely used in production of medicines. The medicinal use of *Calotropis procera* has attained great importance. All the parts of *Calotropis procera* including viz, root, stem, leaf and flowers are in common use for production of useful medicines. The study was designed to analyze and clone the cp1105 gene terminator sequence in expression vector pJIT166. The analysis was performed by using various bioinformatics tools. The cloned fragments were analyzed by running on gel electrophoresis. Further, blue white screening was performed to identify and distinguish the transformants and non transformants.

INCIDENCE OF HEPATITIS B AND RISK FACTORS AMONG UNIVERSITY STUDENTS POPULATION IN STATE OF AJ&K: A CLINICAL STUDY OF HIGHLY EDUCATED COMMUNITY

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Hepatitis B have become very lethal and rapidly increasing infectious diseases of Pakistan. In Pakistan, there are estimated 7-9 million carriers of hepatitis B virus (HBV) with a carrier rate of 3-5%. The study was aimed to determine the incidence of hepatitis B surface antigen (HBsAg) among highly educated community and the risk factors associated with spread of HBV in state of AJ&K, Pakistan. The study was conducted among highly educated community in state of AJ&K, Pakistan. Screening for Hepatitis B surface Antigen (HBsAg) among selected population through Immuno chromatography Kit (ICT) was followed by ELISA for quantification of HBsAg. Viral confirmation and viral load detection was carried out through quantitative real time PCR (RT-qPCR). Demographic information and exposure to expected risk factors from each participant were collected through questionnaire method. Clinical markers such as Hepatitis B envelope Antigen (HBeAg)

detection, Liver Function Tests (LFTs) and Prothrombin Time Test (PT-Test) were performed to facilitate the treatment for HBV positive individuals. A total population of 7015 was screened for HBsAg by using ICT Kit method. 150(2.13%) individuals were found positive for HBsAg after initial screening through ICT Kits. 117(1.7%) individuals were confirmed through ELISA. Out of these 117 ELISA positive individuals, 39(0.55%) were found positive after Quantitative Real Time PCR which include 32(0.46%) male and 07(0.09%) female. Out of 39 PCR positive individuals 30(0.42%) HBV positive individuals were found in the high risk group having viral load above 20,000 IU/mL. 21(52.9%) individuals were found abnormal for LFTS and they had Bilirubin, ALT & Alkaline Phosphate level above the normal range. During the present study we found only 02(5.1%) individuals out of 39 HBV positive individuals which has higher PT or INR range. No individual was found with fatty and enlarged liver during abdominal ultrasound. 2.13% prevalence of hepatitis B showing a great concern regarding prevalence of hepatitis B among the highly educated community and it is an alarming health issue. Awareness of preventive measurements about hepatitis B is the only way to reduce this rate. Better understanding about life cycle of HBV can help us to control the virus multiplication within the host and will surely improve the treatment methodology and immunization against HBV.

AMELIORATING ROLE OF METHANOLIC LEAVES EXTRACT OF *FRAXINUS XANTHOXYLOIDES* AGAINST CCL₄-CHALLENGED NEPHROTOXICITY IN RATS

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Root bark, stem/twigs, and leaves of *Fraxinus xanthoxyloides* are being used regionally for the cure of malaria, jaundice, internal injuries, pneumonia, pain, rheumatism and also in fracture of bones. Our objective was to assess the methanolic leaves extract of *F. xanthoxyloides* for its defensive capability against renal injuries stimulated by CCl₄ in rats. Dried leaves were grinded and extracted with methanol (FXM). Sprague Dawley male rats (48) were equally partitioned into 8 groups. Group I–II: untreated control and vehicle control; Group III: CCl₄ treated at a concentration of 1 ml/kg body weight (bw) with 15 doses given for 1 month. Group IV: silymarin treated (100 mg/kg bw); Group V–VI: FXM administered (200, 400 mg/kg bw) and CCl₄; Group VII–VIII: FXM (200, 400 mg/kg bw) alone in 15 dosages. After duration of 30 days urine was assessed for kidney function, renal tissues for anti-oxidant enzymes, biochemical markers, comet assay, and histology. Enhanced urinary creatinine, urobilinogen levels whereas decreased creatinine clearance, protein contents, and albumin were observed by CCl₄ administration as correlated to control group. CCl₄ injection also decrease the level of GSH, CAT, SOD, POD, GST, GSR, and tissue protein while elevated the levels of TBARS, DNA damages and H₂O₂ in renal tissues of experimental animal. In comparison, co-treatment of FXM and silymarin result in restoration of antioxidant, histopathological and biochemical profiles of kidney. Through this study we affirmed the ameliorating role of *F. xanthoxyloides* in oxidative stress affiliated disorders of kidney.

**ENVIRONMENTAL ECOLOGY OF LESSER GOLDEN BACKED WOODPECKER
DINOPIUM BENGHALENSE IN DISTRICT JAMSHORO, SINDH**

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Members of this family are mainly identified for their characteristics, behavior of mostly foraging on the insects available on the ground and also on the branches of trees. They often communicate by audible drumming on the trees. Woodpeckers have quite brightly colored feathers although the exact colors of the

woodpeckers are variable in different species. Lesser Golden backed woodpecker also known as black rumped flame back woodpecker is a shy bird. Due to its golden and red color the woodpecker can be clearly identified in woods and its inhabiting areas. They have a specified peculiar type of flying pattern by which they can be recognized. They are generally seen singly or in pairs in the forest areas and in open wooded land sides. Its diet includes beetles, insects, ants and pulp of ripe fruit and also flower nectar. Their breeding season starts from Feb to Aug and nesting season varies from March to August. They make their nests in the tree branches. Eggs color is glossy white. Their behavior during breeding season is that they frequently keep on drumming with their beak on the trees. They are identified by their best pecking ability and beautiful colors. The morphological characters of these specie *Dinopium bengalines* are they have quite bright colors including greenish yellow on their upper side of the body mixing with darker brown and blackish in color leading till the end of feathers rest of body is black and white spotted. They have reddish crown also which is different in male and female. The males can be differentiated from females by the entire crown and crest crimson. In males the red color in its crown extends to its forehead, black and white spots are absent in it having black fronted crown merging with black color only while as in females the red color in its crown doesn't reach forward till its forehead and there are clear black and white spots on the front forehead of female so the females differs from males in having the front half of the crown blackish and the tip of each crown feather with the whitish color. They have great economic importance in controlling pest population. The pest which is causing very much harm to agricultural fields. As they feed on insects, larvae and caterpillars so in this way they save the fertility of soil and may cause damage to crops, fruits and trees. I am working on this species and have collected some of specimens for the research work and measurement of different parameters and for identification, different articles and literature is cited including national and international.

DEVELOPMENT OF COTTON LEAF CURL VIRUS ON TRANSGENIC COTTON Cry1Ac

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Cotton is the major cash crop of Pakistan. There are number of insect pests attack on cotton but whitefly is now becoming the major pest of cotton crop which results in declining the yield of cotton from last decades. The experiment was conducted at Cotton Research Institute (CRI) Multan. Three different varieties FH-Lalazar, FH-142 and IUB-2013 were grown in RCBD with five treatments and three replications. Different control measures were applied and checked the infestation of whitefly in all three varieties. Whitefly adults were released in control plot and checked the symptom of CLCuV caused by a vector *Bemisia tabaci* (Genn). Upward curling, stunting growth, dwarfing, vein clearing, vein swelling, cup shape appearance of cotton leaf and leaf enation were recorded. The result showed that there was 57% loss caused by CLCuV in control plot and 10% on each variety. Upward curling, stunting growth, dwarfing, vein clearing, vein swelling and cup shape appearance of CLCuV were recorded but there was no symptom available of leaf enation recorded in all three varieties. It is the need of time to save cotton from whitefly because it is the vector of CLCuV.

TISSUE SPECIFICITY AND STRENGTH OF COTTON FIBER GENES

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Promoters are unique stretches of DNA sequences that regulate the transcriptional control of gene expression. Promoters have various regulatory motifs crucial for expression specificity. Promoter can be

divided into constitutive, tissue specific or inducible promoters depending upon mode of expression. This is interplay of *cis* acting element located on promoter and transcription factors. Cotton fiber promoter are of great importance in biotechnology as these may be used to express fiber genes in cotton specifically. Although some of cotton fiber gene promoters show expression in fibers but most of these promoters also expression various other organs like trichomes, pedicles and vascular tissues. Due to difficulties in somatic embryogenesis in cotton these promoters are mostly studied in heterologous systems like Arabidopsis and tobacco. In this article, we have reviewed expression of promoters of some cotton fiber genes. These promoters may be used to express foreign genes in cotton fibers specifically as well as in dicots.

QUANTITATIVE EXPRESSION STUDY OF SOME FIBER GENES IN SHORT AND MEDIUM FIBER LENGTH GENOTYPES OF COTTON (*GOSSYPIMUM HIRSUTUM* L.)

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Cotton fibers are unicellular trichomes arise from ovule epithelial cells. A large number of genes are involved in the process of fiber development. Expression pattern of five cotton fiber gene families was determined in short fiber length genotype of cotton through real time qPCR. Expression analysis revealed that transcripts of expansin, tubulin and E6 were elevated from 5 to 20 days post anthesis (DPA) in fibers. Three Lipid transfer proteins (LTPs) including LTP1, LTP3, LTP7 exhibited highest expression in 10 - 20 DPA fibers. Transcripts of LTP3 were detected in fibers and non fiber tissues while that of LTP7 were almost negligible in non fiber tissues. Sucrose phosphate synthase gene showed highest expression in 10 DPA fibers while sucrose synthase (susy) expressed at higher rate in 5-20 DPA fibers as well as roots. The results demonstrated that 5-20 DPA fibers showed high expression of most of genes. The study provides an important insight into gene expression in short fiber cotton line and relation of fiber length with gene expression.

IMMOBILIZE A WOLF: PROTOCOL, SAMPLING AND MORPHOMETRIC DATA COLLECTION

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As a part of ongoing PhD project a safe and easy protocol is designed to immobilize the grey wolf to get blood samples for phylogenetic analysis along with different 30 morphometric measurement for their characterization in Pakistan. Three sets of doses varying from 5.0-10.5 mg/kg of Zoletil® 50 (125 mg Zolazepam base & 125 mg Tiltamine base) will be employed on 13 wolves in captive conditions in different Zoos and other animal conservational areas of Pakistan. A time log from Induction to Recumbancy and recovery will be recorded by stop watch to validate the best suitable dose of Zoletil® 50 (125 mg Zolazepam base & 125 mg Tiltamine base) for grey wolf immobilization without stress and capture myopathy. Similarly the important physiologic parameters i.e. respiration rate, body temperature, salivation, capillary reflex time and mucosal color will be recorded after every 10 minutes. The physiologic data collected will be correlated with three sets of doses to know the best one for grey wolf population in Pakistan. The study will enhance the husbandry and handling skills of the zoo and other captive facilities staff in Pakistan to manage properly important carnivores in captivity to further strengthen the breeding programs at national and international level.

SCREENING OF CELLULOSE DEGRADING EXTREMOPHILE ENZYMES FROM INDIGENOUS FUNGI

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Cellulase enzymes obtained from various sources has been widely used for many applications, such as enzymatic scarification of cellulosic materials, deliberations of flavor components in fruit juices and the release of phenolic compounds with antioxidant activity from fruit and vegetable residues. Pakistan is importing about 500 tons of cellulase to meet the demands in the country at an expense of Rs. 100 Million/annum The increased need for considerable Cellulase activity has strongly stimulated this study. About 20 fungi were collected from thermophilic areas of Pakistan including Sibbi, Multan and D.G Khan during summer. These samples were collected from wood, soil and leaves. The soil dilution and soil plate method on media; Potato Dextrose Agar was used as isolation techniques. Screening for cellulase production is done by cellulase plate assay for this *Conidia*, 10⁶/ml, were plated in the centre of Petri dishes containing 1 % CMC agar medium which were flooded with an aqueous solution of Congo red (1% Congo red in distilled water). Zones of CMC hydrolysis is visualized. Seven samples showed positive cellulase activity. Which were morphologically identified as *Aspergillus flavus* and *Aspergillus fumigatus* by microscopy. DNA was extracted by using phenol chloroform isoamyl alcohol method and amplification was done using universal primers (ITS1 and 4) after PCR purification sample are sent for sequencing. The study suggests that Pakistan can fulfill its cellulase needs by using its indigenous source.

STUDY THE GROWTH RATE OF DIFFERENT PLANT SPECIES IN AN AQUAPONIC SYSTEM.

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Aquaponics is the combined culture of fish and hydroponic plants in recirculating systems. It is a very productive and ecologically sound food production system, where fish waste provides a nutrient source for nitrifying bacteria, which in turn convert toxic waste of the fish to useful nutrients for plants. Aquaculture effluent provides most of the nutrients required by plants if the optimum ratio between daily feed input and plant growing area is maintained. An experiment was conducted in laboratory. The objective of this research includes the operation of an aquaponic system for producing marketable fish and different plant species. The system consist of two aquariums and pump which recirculate the water from fish tank to plants which are soil less plants. The feed ratio of fish in aquarium A1 is about 2 times and A2 is about 4 times per day. Plants are soilless so it get nutrients from fish waste ammonia and in return plants filter the water for fishes and maintained the water quality both fishes and plants are symbiotic to each other. The production of Different plant species *Capsicum frutescens*(Carl linnaeus) which is commonly called hari mirch , *Mintha arvensis* (Carl Linnaeus) commonly called dhaniya, *Capsicum annum* (Carl linnaeus) commonly called shimla mirch, Ornamental plants like *Variegatum* (Andrien) commonly called Spider plant and *Epipremnum aureum* (Linden and Andre) commonly called money plant as well as *Cyprinus carpio* grow at higher rate in A2 in which feed ratio is of 4 times per day as compared to A1 in which feed ratio is of 2 times per day. Aquaponic technique is capable of producing 5000 kg of vegetables and 500 kg of fish per year by utilizing limited space. Aquaponics is a biointegrated food production system that links recirculating aquaculture with hydroponic vegetable, flower, and herb production. Due to limited resource of fresh water and soil in urban environment aquaponics technique is a very efficient production system for both fish and vegetables..No pesticides or antibiotics are used at any stage therefore; the aquaponic production system can be regarded as a part of the organic agriculture.

ASSESSMENT OF THE EFFECT OF DETERGENT INDUCED TOXICITY ON BEHAVIOR AND HISTOLOGY OF ROHU (*LABEO ROHITA*)

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The current study was conducted to investigate the behavioral, morphological and histopathological changes in Indian Major Carp (*Labeo rohita*) exposed to sub lethal concentration of detergent (Surf excel). The fish were exposed to 20mg/liter for 45 days with a simultaneous control group. The behavioral changes observed during the experiment were loss of appetite, loss of mucus, frequent surface to bottom movement, restless condition, loss of equilibrium, sinking to the bottom and respiratory distress. In some fish bulging out of eyes was a noticeable morphological change observed during the research. Sampling was conducted after every 15 days from each treated and control group. Results of first fortnight manifested the appearance of curling of secondary gill lamellae, fusion of secondary lamellae, mild atrophy of primary gill lamellae, mild congestion of blood space and slight epithelial lifting in the gills section whereas in liver minor congestion and slight necrosis was identified. Similarly commencement of vacuolization process was also evident in the cytoplasm after first 15 days. Succeeding, displacement of the epithelial layer of the secondary lamellae, fusion and curling of secondary lamellae, oedematous condition, and congestion of blood vessels was perceived in the gill sections of 30 days while in the liver congestion at the sinusoids, necrosis, pyknosis, hyperplasia and vacuolar degeneration was identified. Similarly, after 45 days of treatment the histological observation of gills revealed an extreme condition of epithelial lifting, curling, fusion and disruption of secondary lamellae. Further chronic oedematous condition and congestion of blood vessels was observed in the section of gills. However in liver, severe congestion of blood vessels, vacuolar degeneration and severe necrosis was prominent. It is concluded that Surf excel is highly toxic, even in sub lethal concentrations, for the health and survival of fish.

VARIABILITY IN MUNGBEAN GENOTYPES FOR RESISTANT TO *CALLOSobruchus MACULATUS* (F.) UNDER LABORATORY CONDITION

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Seed yield losses in mungbean during storage due to bruchids (seed beetles) is a serious problem for farmers, consumers, seed companies and traders. Bruchids beetles are the principal post-harvest pests of stored mungbean. High infestation during moon soon season renders grains totally unfit for human consumption and maximum adult emergence leads to complete destruction of stored experimental breeding material. Bruchid beetle infestation is a major contributor to quality deterioration of stored mungbean seed kept in warm and humid conditions. Damaged grains cannot be utilized for agricultural and commercial purposes. Bruchids infestation of mungbean grains can decimate a farmers output and therefore, they have to sell mungbean immediately after harvest, accepting any price that is offered. In storage, the adult female lay eggs directly on seed coat. The newly hatched larva bore through the egg shell and penetrates seed coat, continue to feed and complete their development inside the seed. After completion, the insects emerge as adult beetles leaving behind a hole at the exit point. The alternative to chemicals and other control measures is to develop bruchids resistant genotypes required for storage purposes. The entomological research on bruchids is aimed at screening progenies obtained from crosses made by Mungbean Breeder for resistance/tolerance to beetles. Under joint coordinated research project with Mungbean Breeding Group at NIFA on "Breeding for Bruchid Resistance in Mungbean" research work was carried out with an objective to identify bruchid resistant genotypes and incorporate resistant genes in local high yielding well adapted genotype. Culture of bruchids beetle was maintained on bold mungbean grains at 28 ± 2°C and 70 ± 5% relative humidity. Adults of uniform age males and females were collected separately by isolating mungbean grains in small transparent glass test tubes with mouth plugged with cotton. The stock culture maintained was utilized for conducting the experiment. Four mungbean genotypes viz. V2709, V2802, V1128 and V2817 were evaluated to ascertain their resistance to

bruchid beetles. The resistance of mungbean genotypes was evaluated on the basis of grain infestation, % grain damage, % adult emergence & developmental period. The results showed that among the tested genotypes, minimum grain damage of 24.3% was recorded in V2817 having developmental period of 21 days with 13.6% adult emergence. Maximum grain damage of 43.6% was recorded in genotypes V1128 followed by V2709 (35.5%) with mean developmental period of 21 days & adult emergence of 23.9% and 18.5% respectively. The conclusion is that genotype found tolerant to bruchid will be utilized in mungbean breeding program for incorporation of the desired resistant characters in the local adapted mungbean genotypes.

PESTICIDES ACUTE POISONING, SUICIDES AND DISABILITY IN HUMANS

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Pakistan is a place where only pesticides is considered as an effective source for control of pests and diseases, and we are using these deliberately by involving wide range of pesticides. Majority of chemicals are used in agriculture as data suggests that about 49399 million metric tons of pesticides had been used only in agriculture sector in 2010 in the country. Use of pesticides in other fields such as public health, fisheries, forestry and food industry is not included in the data. Though pesticides are useful in managing pest populations but these have adverse effects on the humans, animals and ecosystems. The current work exhibits the effects of pesticides on human health in the country. Acute poisoning is one of the key issues resulted by the unsafe handling and storage of pesticides in on-farm and off-farm premises in developing economies. From various areas in the country, pesticides are reported to be used as tool for suicide by teen agers due to easy access. According to the department of Plant Protection (Karachi), total of 200 workers get affected showing the low enzyme activity in their blood. In Multan, an adverse situation was observed as elevated levels (87.5 %) of AchE activity was recorded in female cotton pickers as compared to males which have low (36 %) AchE activity because cotton is picked mostly by women in Pakistan. The aim of this work is to sum-up the weaknesses in practicing / implementing current policies and legislations for safe handling, storage and judicious use of pesticides. The acquired knowledge will later be used in making and improving the existing regulations for the subject.

SECTION – II

PESTS AND PEST CONTROL

PESTS CONTROL METHOD OPTED BY LOW INPUT SUGARCANE GROWERS IN TWO
SELECTED DISTRICTS OF KHYBER PAKHTUNKHWA, PAKISTANFaheem Khan^{1*}, Zelle Huma², and Ahmad-Ur-Rahman Saljoqi²¹ Department of Agricultural Extension Education and Communication, The University of Agriculture,
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This research was conducted to strategies opted by low input sugarcane (*Saccharum officinarum*) growers against pests' infestation in the Mardan and Charsadda districts of Khyber Pakhtunkhwa, Pakistan. The principal researcher interviewed face by face three hundred and thirty-six sugarcane growers using a well-designed and pre-tested interview schedule. These sugarcane growers were selected using a multi-stage sampling method. Most growers reported *Rats*, *Termites*, *Borers*, and *Leafhoppers* as the main pests in their sugarcane crops. Results shows that sugarcane growers mainly applied pesticides including *Rigid* (*Fipronil*), *Malathion*, *Furadon* (*Carbofuran*) and *Imidacloprid* against the previously mentioned pests to decrease or eliminate their infestations. Those sugarcane growers who applied pesticides achieved average sugarcane average yields up to 105.57 t ha⁻¹, while those sugarcane growers who did not treat these pests achieved averaged yields below the 63.39 t ha⁻¹. It is concluded that government bodies and other concerned organisations should create awareness about pests' infestations and provide training regarding control method for pests in sugarcane field. Besides these suggestions, the government ought to ensure availability of pesticides in the local market at the right time and at a reasonable price.

IMPROVING APPEALING TENDENCY OF METHYL EUGENOL BAIT
AGAINST *BACTROCERA ZONATA* IN MANGO ORCHARDTaimur ur Rehman¹, Fahad Nazir Khoso^{1*}, Muzaffar Ali Talpur², Imran Khatri¹,
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Tephritid flies are the most destructive pests of fruit crops throughout the world especially in Asia and Africa. Among these flies, *Bactrocera zonata* is the significant pest in Pakistan and India. Growers have experimented many control measures to reduce fruit fly infestation but nowadays most common and effective practice is Male Annihilation Technique (MAT) by using Methyl Eugenol (ME) as male attractant. Present research was carried out to improve the efficacy of this technique by using ME in combination with mango essence and Ethanol. An experiment was carried out from May 2017 to August 2017 in Latif mango farm, Tandojam, Pakistan. The traps were installed in the orchard of Dasehri variety. Five treatments were tested, in which one Treatment kept control (T5) having ME 4ml (Methyl Eugenol 85% + sugar solution 10% + insecticide 5%). In rest of the traps, two wicks were used one with same as control and other with 4 ml of Mango Essence combined with Ethanol percentage. T1 was ME 4 ml + mango essence 4 ml, T2 was ME 4ml + Essence 4ml with Ethanol 5%, T3 was ME 4ml + Essence 4ml with Ethanol 10%, T4 was ME 4ml + Essence with Ethanol 15%. The results of the study revealed that the highest number of flies (1849.30±35.42) was captured in the month of August, whereas; lowest number (147.84±2.17) was collected in the month of May. Furthermore, findings of the experiment showed that highest flies were observed in the trap containing T3 (sum of means, 4418.32±18.63), while T5 (sum of means, 2850.58±13.73) captured the lowest numbers of *B. zonata*. All the treatments had strong negative relationship with temperature, whereas; humidity had positive effect on

treatments, while wind speed caused negative influence on flies. It was concluded that T3 (ME solution+ essence+ Ethanol 10%) was more effective than other treatments experimented, whereas; temperature had negative impact on all the treatments prepared while humidity had positive effect.

BIOCHEMICAL VALIDATION AND EFFICACY ENHANCEMENT OF NIFA, BIOLARVICIDE FOR MOSQUITO VECTOR CONTROL (*AEDES AEGYPTI*)

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Experiments were carried out at NIFA to study the biolarvicide activity of NIFA biolarvicide. In this experiment the biochemical validation of paper nigram was conformed. The previous experiment data of THAIR BADSHAHA (UNPUBLISHED DATA) has a close similarity with our data. This experiment a synergistic combination of paper nigram biolarvicide and that of harmala was developed. A first experiment harmala extract was used as larvicide at concentrations of 23ppm and 100% mortality was achieved after 72 hours. The paper nigram biolarvicide showed a fast motility of larvae and after 48 hours, all the larvae were found dead in case of paper nigram at 23ppm. Another experiment having synergistic combination of paper nigram with harmala at 20 and 23ppm. It was applied on larvae and complete mortality was observed within 48 hours. It for the full mortality at 23, 21, and 13ppm it took 72 hours. At 10 and 7.5ppm the harmala extract showed full mortality after 96 hours and at 5ppm it took 108 hours full mortality. The biodegradation study was also conducted in this experiment fresh solution of THE PEPER NIGRAM and harmala made and kept for 5 days and trials were taken after 5 days. The harmala 21ppm showed full motility after 144 hours and at 19ppm concentration harmala took 168 hours for full mortality. The synergistic combination of paper nigram with harmala at 21ppm showed full mortality after 60 hours. In another experiment the same above procedure was repeated in this case solution of harmala and paper nigram was kept for 25 days. No mortality was observed in each of the treatments, which showed that this larvicide has been degraded.

THE USE OF NUCLEAR TECHNIQUES IN THE CONTROL OF INSECT PESTS OF QUARANTINE IMPORTANCE

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Citrus and mangoes are the top exporting fruits of Pakistan due to their high acclaimed taste and quality tributes. The citrus psyllids and scale insects are the associated key pests of these fruits in Pakistan and quarantined in many parts of the world. Pakistan consequently loose export of its high quality citrus and mangoes to the pest free countries as the eggs and nymphs of these pests can be transmitted with fresh fruits and nursery plants to the importing countries. The WTO regulations for export of fresh commodities require disinfestations/ irradiation treatment of these quarantine pests. Studies on disinfestation of fruits with gamma irradiation from a Cobalt Co⁶⁰ source as phytosanitary measure for citrus scale *Aonidiella aurantii*, mango's scale *Aspidiotus destructor* and citrus psyllids *Diaphorina citri* was conducted for the first time in Pakistan to investigate mortality and growth inhibition of these pests. Dose response tests were conducted with eggs, 1st, 2nd stage and 3rd stage nymphs, and adult females without eggs and with eggs in a series of radiation doses between 100 and 300 Gy. All life stages of *A. aurantii*, *A. destructor*, and *D. citri*, were affected with gamma irradiation. The pattern of tolerance to irradiation was eggs <1st instar<2nd instar<3rd instar and adults. The adult females of *A. aurantii* did not produce F1 generation at 220 Gy. Similarly, a dose of 200 to 217 Gy was determined to completely stop *A. destructor* and *D. citri* development to subsequent stages. Results from validation tests indicated a dose of 220 Gy. for 99.99% inhibition of *A. aurantii*, *A. destructor*, and *D. citri* pests and therefore, recommended to provide quarantine security to citrus and mangoes fruits from these pests.

TOXIC AND REPELLENT EFFECTS OF EMAMECTIN BENZOATE AND LUFENURON AGAINST *TRIBOLIUM CASTANEUM*

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Red flour beetle, *Tribolium Castaneum* (Herbst) (Coleoptera: Tenebrionidae) cause quantitative and qualitative damage to cereals and other stored grains. *T. castaneum* feeds on the grain and cause significant reduction in weight. Qualitative damage is due to product alterations such as loss of nutritional and aesthetic value, and loss of industrial characteristics such as baking. Control of *T. Castaneum* through synthetic chemicals is common especially in stored grains. In this study, we used two different synthetic chemicals namely emamectin benzoate and lufenuron for the control of *T. castaneum*. Bioassay results showed 100% adult and larval mortality of *T. castaneum* by (0.2%) concentration of emamectin benzoate and lufenuron. Filter paper-based free choice tests showed maximum repellency of *T. castaneum* by emamectin benzoate, repelling 63.33% adults and 68.33% larvae of *T. castaneum*, compared with 66.66% adult and 65% larval repellency by lufenuron. This indicates that emamectin benzoate and lufenuron have better potential to control *T. castaneum* in stored grains.

DIVERSITY, ABUNDANCE AND SPECIES COMPOSITION OF COMMENSAL RODENTS IN DISTRICT BAGH

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The study was conducted from January 2016 to January 2017 in urban areas of district (Bagh). Three areas were selected (Mohala Khawajan, Qandeel Colony, Urja) of (distt Bagh) for sampling of rodents. At each study site, six houses, three shops and one farm house were randomly selected for trapping of rodents. A total of 628 rodents and shrews were captured during 1200 trap nights, with a 0.52 captures per night. The overall trap success remained 52.3%. The rodent abundance index was also calculated to find population dynamics. The indexes were found greater than 1 and recommend one trapping treatment of seven days at mid of each season.

SUGARCANE LEAFHOPPERS OF TANDOJAM

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For present studies specimens of leafhoppers were collected from sugarcane crop at Tandojam. Further examination and identification was carried out at insect systematic laboratory, department of Entomology, Sindh Agriculture University Tandojam. Collection was made through sweep net and pooter. To identify the specimen up to the species level, keys for the region were collected from various publications. In present study total 135 members of the family cicadellidae were collected from sugarcane crop. During the course of identification it revealed the occurrence of 10 species of under two subfamilies; 9 species under Deltocephalinae and 1 species under Cicadellinae. Subfamily Deltocephalinae exposed 6 tribes, tribe: Scaphytopiini Oman, 1943 with one species *Varta rubrofasciata* Distant 1908; Macrostelini, Kirkaldy 1906 with two species records *Balclutha rubrostriata* (Melichar, 1903) and *Balclutha incisa* (Matsumura, 1902); tribe Goniagnathini, Wagner 1951 with one species *Goniagnathus guttulinervis* (Kirschbaum 1868); tribe Hecalini Distant, 1908 with two species *Hecalusindhensis* (Ahmad & Aziz, 1988) and *Hecalus porrectus* (Walker, 1858); tribe Stenometopiini, Baker 1923 with one species *Stirellus lahorensis* (Distant, 1918); lastly tribe Chiasmini, Distant 1908 revealed two species *Exitianus indicus* (Distant, 1908) and *Exitianus nanus* (Distant, 1908). Further subfamily Cicadellinae, Latreille 1825, tribe Cicadellini revealed the occurrence of single species

record *Cofana spectra* (Distant, 1908). 38th congress of zoology (International) February 27 – March 1, 2018
Department of Zoology, University of the Punjab Lahore.

SIGNIFICANCE OF LEAF BEETLES (CHRYSEMELIDAE) IN AGRICULTURE

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Leaf beetles are the members of family chrysomelidae, many play an important role either as a pest or as a biological control agents of weeds, both adults and grubs cause losses to crops, trees, medicinal plants (Mirozoeva, 2001), flowers (Jolivet and Hawkeswood, 1995), they play an important role in dissemination of plant diseases (Walters, 1969). Family chrysomelidae consists of 60,000-70,000 species (Jolivet et al. 1988). The subfamilies which are recognized as harboring pest leaf beetles include; chrysomelinae criocerinae, galerucinae, eumolpinae, alticinae and hispinae. Chrysomelidae fauna in Pakistan is relatively poorly studied, some of the publications can be seen that have a certain economic importance. The leaf beetles known to occur as a pest in Pakistan include; red pumpkin beetle found commonly on several crops, estimated losses to crop reaches 30-100% (Adam, 1969), its polyphagous behavior expanded its host range till 81 plant species; pumpkin, wax gourd, water melon, cucumber, bottle gourd and many fruit crops (Butani and Jotwani, 1984). Hispa *Dicladispa armigera* is one of the notorious pests of rice crop in India and Pakistan, it causes loss to both deep water and upland rice (Prakasa Rao et al., 1971; Budhraj et al., 1979); and cause 35-65% grain loss (Hazarika et al. 2005). Seed beetles are the members of subfamily Bruchinae, they are recognized as pests of mainly leguminosae, but they also cause damage to other plants (Johnson, 1990; Kergoat et al., 2008). Several flea beetles are known to feed on sweet potato including; *Systema blanda*, *Systema elongate*, *Chaetocnema confinis*, *Diabrotica balteata* and *Diabrotica undecimpunctata howardii*. In Punjab *Parthenium hysterophorus* L. is considered as a noxious weed, larvae and adult of Mexican beetle *Zygogramma bicolorata* Pallister were found feeding on leaves of this weed, and the beetle can be used as a biological control agent in Pakistan. There is a great need of identifying chrysomelid fauna of Pakistan so that important species can be separated from less important ones. 38th congress of zoology (International) February 27 – March 1, 2018 Department of Zoology, University of the Punjab Lahore

TAXONOMIC AND ECOLOGICAL STUDIES OF ARANEID FAUNA FROM GUAVA GARDENS OF TEHSIL (JARANWALA AND SUMUNDARI) DISTRICT FAISALABAD PAKISTAN

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Spiders play an important role as biological control agent. The present study was conducted from April to October for the evaluation of Taxonomy and ecological parameters of spider fauna from two Tehsils of district Faisalabad. There are many factors which are causing the biodiversity loss as high temperature and habitat loss. The biodiversity of spiders was assessed from different guava gardens with the help of Hand picking, Jerking and Pitfall trap methods. Samples were collected in 70% alcohol in small glass bottles and identified in the Zoological Laboratory of the University of Sargodha Women Campus Faisalabad. A total of 562 specimens belonging to 7 families, 10 genera and 21 species were recorded. Family Lycosidae was the most dominant with 304 specimens belonged to 9 species while Gnaphosidae (9), Oxyopidae (11), Clubionidae (11) and Araneidae (12) with two, one, one and one species respectively were least common of all the families. Maximum spiders (146) were captured in the month of June with average rainfall, temperature and relative humidity 65.5 mm, 33.65 °C and 71.4 % respectively followed by May (100) and April (93). Among the 21 species of spiders collected during sampling had common occurrence in both Habitats while *Lycosa rori*, *Oxyopes ratnae* and *Clubiona* species were found only in Habitat-1. Ecological parameters i.e. Shannon diversity index (H),

Simpson's diversity Index (D), species richness (R) and Pielou's Evenness Index (E) were 2.89, 0.94, 77.99 and 0.94 at Habitat-I and 2.87, 0.94, 62.61 and 0.94 at Habitat-II respectively.

COMPARATIVE EFFICACY OF BIOSYNTHESIZED AND CHEMOSYNTHESIZED NANOPARTICLES IN MOSQUITO CONTROL AT LAHORE, PAKISTAN

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Mosquitoes act as vectors in transmitting various diseases like dengue, malaria, filariasis, Japanese encephalitis. Due to insecticide resistance and effects on non-target organisms, there is a need of time to develop improved, new and more effective but economical mosquito control methods. Present study was aimed to test the efficacy of silver nanoparticles (AgNps) and zinc oxide nanoparticles (ZnONps) in mosquito's control. AgNps were prepared from aqueous leaf extract of a plant *Azadirachta indica*. ZnONps were synthesized from Zinc chloride and sodium hydroxide solutions against three common species *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Prepared nanoparticles were characterized by using UV-visible spectroscopy and X-Ray Diffraction (XRD) techniques. Mosquitoes' larvae (1st, 2nd, 3rd and 4th instars) were exposed to 0.5mg/L of AgNp's and ZnONps and mortality rate was recorded at different time intervals i.e., 12 hours, 24 hours, 30 hours, 48 hours, 72 hours, 96 hours and 120 hours. Results indicated that AgNps and ZnONps exhibited 100% larvicidal activity. ZnONps were most promising against 2nd instar of *Cx. quinquefasciatus* with LT₅₀ and LT₉₀ values of 15.7 and 26.5 respectively. AgNps were also very effective against 2nd instar of *Ae. aegypti* with LT₅₀ and LT₉₀ values of 74.4 and 102.9 respectively. It was evident from the results that these nanoparticles are most eminent biolarvicidal agents for mosquito control with no hazardous effects on non-target individuals while chemically synthesized nanoparticle shows efficient result but it have drastic effects on environment.

IMPROVING APPEALING TENDENCY OF METHYL EUGENOL BAIT AGAINST *BACTROCERA ZONATA* IN MANGO ORCHARD

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Tephritid flies are the most destructive pests of fruit crops throughout the world especially in Asia and Africa. Among these flies, *Bactrocera zonata* is the significant pest in Pakistan and India. Growers have experimented many control measures to reduce fruit fly infestation but nowadays most common and effective practice is Male Annihilation Technique (MAT) by using Methyl Eugenol (ME) as male attractant. Present research was carried out to improve the efficacy of this technique by using ME in combination with mango essence and Ethanol. An experiment was carried out from May 2017 to August 2017 in Latif mango farm, Tandojam, Pakistan. The traps were installed in the orchard of Dasehri variety. Five treatments were tested, in which one Treatment kept control (T5) having ME 4ml (Methyl Eugenol 85% + sugar solution 10% + insecticide 5%). In rest of the traps, two wicks were used one with same as control and other with 4 ml of Mango Essence combined with Ethanol percentage. T1 was ME 4 ml + mango essence 4 ml, T2 was ME 4ml + Essence 4ml with Ethanol 5%, T3 was ME 4ml + Essence 4ml with Ethanol 10%, T4 was ME 4ml + Essence with Ethanol 15%. The results of the study revealed that the highest number of flies (1849.30±35.42) was captured in the month of August, whereas; lowest number (147.84±2.17) was collected in the month of May. Furthermore, findings of the experiment showed that highest flies were observed in the trap containing T3 (sum of means, 4418.32±18.63), while T5 (sum of means, 2850.58±13.73) captured the lowest numbers of *B. zonata*. All the treatments had strong negative relationship with temperature, whereas; humidity had positive effect on

treatments, while wind speed caused negative influence on flies. It was concluded that T3 (ME solution+ essence+ Ethanol 10%) was more effective than other treatments experimented, whereas; temperature had negative impact on all the treatments prepared while humidity had positive effect.

RESPONSE OF TWO DIATOMACEOUS EARTH FORMULATIONS AS GRAIN PROTECTANTS AGAINST *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRYCHIDAE)

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In this experimental bioassay, the efficacy of diatomaceous earth (DE) as two DE commercial formulations was evaluated against *Rhyzopertha dominica* (F.) (Coleoptera; Bostrychidae). In the experiment, three doses (300, 600 and 900 ppm) of two commercial DEs (SilicoSec and PyriSec) were tested against adults of *R. dominica* in wheat. Mortality of the exposed adults in experiment was assessed after 5, 10 and 15 days of exposure. The most effective DE was PyriSec which caused complete mortality of *R. dominica* adults at 300 ppm after 5 and 10 days of exposure in wheat. SilicoSec DE caused low mortality (< 25%) even after 15 days of exposure at the highest dose. Results from SilicoSec in the experiment indicated that mortality levels for *R. dominica* was high, but only at 900 ppm. The increase of temperature increased the efficacy of the tested DEs against adults of *R. dominica*. Higher mortality levels were recorded in the low RH. The PyriSec was more effective as compared with SilicoSec against *R. dominica* adults at 900 ppm and 25°C.

INFESTATION OF MAIZE BORER ON DIFFERENT VARIETIES OF MAIZE

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Maize stem borer (*Chilo partellus*) (Swinhoe) (Lepidoptera: Pyralidae) is a common pest of maize. It causes serious damage to the maize crop. It also causes solemn damage on different parts of the plant. Different local varieties of maize were screened to check the early infestation by maize stem borer. Seven maize varieties viz., C2P-132001, FH-948, MMRI-yellow, Pearl, Sadaf, Sahiwal-2002 and YH - 1899 were obtained from Maize and Millets Research Institute, Yusafwala, Sahiwal to check the relative resistance against maize stem borer. Research was done on experimental field area of University College of Agriculture and Environmental Sciences, the Islamia University of Bahawalpur following Randomized Complete Block Design with three replications. The total area selected for research was 35 x 70 ft². Maize was planted with row to row and plant to plant distance maintained as 75 cm and 23 cm respectively. After a few weeks of planting maize in the field, data regarding dead hearts, leaf holes, larval population and larval excreta was recorded on weekly basis. Results on these varieties for early infestation for these parameters revealed that variety C2P-13-2001 showed minimum dead hearts and was proved resistant while variety YH-1899 showed maximum dead hearts by borers. In case of damage in terms of leaf holes the variety YH - 1899 proved resistant compared to the Sadaf variety. FH-948 was more resistant than MMRI-yellow for the larval excreta. Larval population was more on YH-1899 and less on C2P-13 2001. Seasonal infestation results showed dead hearts were present more on date 25-05-2017 and less on 27-04-2017 and the larval excreta was found more on 27-04-2017 and less on 11-04-2017. These data are important for further screening of these varieties for management of maize stem borer.

VARIETY AND PLANTING DATE EFFECTS ON THE INCIDENCE OF BOLLWORMS AND SUCKING INSECT PESTS OF COTTON (*GOSSYPIMUM HIRSUTUM* L.)

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Cotton plays a vital role in Pakistan agro-based economy and is a major source of foreign exchange earnings. It is attacked by several insect pests which limit its productivity. The experiment was conducted in split plot factorial design at Nuclear Institute of Agriculture (NIA) Tandojam to determine the appropriate time of sowing in respect to the attack of sucking and bollworm insect pest complexes and yield of fifteen cotton varieties. The varieties were sown on three different dates (31st March, 20th April and 10th May) representing early, mid-season and late planting times, respectively. The results indicated that the two variables (planting dates and varieties) had pronounced effect on the infestation of insect pests. The March, 31st (early sowing) harbored the lowest seasonal mean infestation of Jassid (0.29/leaf), thrips (1.63/leaf), whitefly (0.45/leaf), pink bollworm (1.92%) and spotted bollworm (3.28%) as compared to other two planting dates. Maximum seed cotton yield was also recorded in early sown crop (2119 kg/ha) followed by mid-season planting (2041kg/ha). Among different varieties, IR-NIBGE 901 and IR-NIBGE 3701 proved to be the most efficient rendering the lowest infestation of insect pests with highest recorded yield of 2637 kg/ha and 2480 kg/ha, respectively. Thus, it is concluded that the pest damage on cotton crop can be minimized by manipulating planting dates.

MONITORING RESISTANCE OF *PHENACOCCLUS SOLENOPSIS* TO NOVEL INSECTICIDES IN SELECTED AREAS OF PUNJAB, PAKISTAN

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The mealybug, *Phenacoccus solenopsis* Tinsley is one of the most detrimental, invasive, and polyphagous pest of cotton which devastate cotton quality as well as quantity and cause economic losses. In Pakistan a number of insecticides are being used to manage this pest. *P. solenopsis* developed insecticides resistance. Consequently, purpose of the present study is to come across the toxicity and status of resistance to recently introduced new chemical insecticides (i.e. spirotetramat, spiromesifen and pymetrozine) in *P. solenopsis*. For this purpose, populations of *P. solenopsis* were collected from two changed locations i.e. Bahawalpur and Multan and reared under laboratory conditions to gain homogenous population. Second instar nymphs were exposed to insecticides by means of leaf dip method. The RRs were in range of 39.27 to 42.65-fold for spirotetramat, 35.19 to 48.82-fold for spiromesifen, and 21.23 to 32.80-fold for pymetrozine in unlike populations of *P. solenopsis* compared to the susceptible population. The integrated resistance management plans like careful use of insecticides with precise application rate and methods, rotation of insecticides and bio control apparatuses must be employed to prevent the control failures, those areas where *P. solenopsis* has developed resistance to the tested insecticides.

FLEAS (SIPHONAPTERA) INFESTING COMMENSAL RATS TRAPS FROM POULTRY FARMS IN SINDH

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A total of Ninety Five rats (*Rattus rattus* and *Rattus norvegicus*) were trapped from different poultry farms of three poultry estates of Karachi district. These rats were brought to the Medical Zoology Laboratory,

VPCI, SARC. All rats were anaesthetized, the fur of the rats was carefully brushed/or blown and examined for fleas, out of 95 rats 51 rats were infested with 90 specimens of fleas. The fleas thus recovered were collected, fixed in 70% alcohol and placed in numbered vials. The fleas were macerated in a solution of 10% KOH. The specimens were kept in this solution for about 24-72 hours. All these specimens were processed through standard procedure, permanently mounted and after morphotaxonomic studies, these were identified as 2 genera and 3 species namely *Xenopsylla astia*, *Xenopsylla cheopis* and *Synostermus cleopatrae*. Fleas were collected from infested rats, the dominant species of flea was *X. astia*. The second dominant species was *X. cheopis*, *S. cleopatrae* was too small to reveal the phenology of their occurrence. *R. rattus* and *R. norvegicus* are commonly regarded to be the usual flea carrier and possible hosts incriminated in spreading of plague and other murine diseases.

PREFERENCE OF FRUIT FLY ON DIFFERENT HEIGHT OF MANGO FRUIT FROM GROUND

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Mango fruit fly *Bactrocera zonata* (Tephritidae; Diptera) is a solemn pest of mango fruit. It causes severe damage to mango fruits in Pakistan. There are total 651 described species while among them 50 have been documented to cause negative impact on economy and decreases its international value. Beside attacking in the field, it also infests rotten dropped fruits To observe the preference of fruit fly on mango fruit height from the ground. The experiment was performed in Moza Buch Mubarak, Multan. We select the plants of mango with different fruit heights from the ground viz, 0 feet, 1 feet, 2 feet, 3-5 feet and 5-6 feet. On daily basis, the % of infestation was recorded on 0 feet, 1 feet, 2 feet, 3-5 feet and 5-6 feet fruit height respectively. After our observations, it is revealed that there is high infestation percentage (20%) of fruit fly on fruits with height 5-6, followed by 3-5 feet (14%), 2 feet (8%), 1 feet (3%) and 0 feet (2%).

INTEGRATED MANAGEMENT OF PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) AND WHITEFLY, *BEMISIA TABACI* (GENNADIUS) ON COTTON CROP

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Farmers are discouraged to grow cotton due to difficulties in managing pink bollworm and whitefly. The yield losses in cotton from these two pests are high for the last 5-6 years. Consequently, the plant protection cost has significantly increased and farmer's profitability has declined who have been growing cotton for generations have already switched over to alternate crops or seriously thinking to do so. Under such conditions a sound IPM programme is the only way forward. There is no hard and fast structure of an IPM programme which could fit in to any agro-eco system. IPM programmes are designed to local situation by integrating available tools and techniques in a compatible way to devise a sustainable pest management programme. An IPM programme was designed and practiced on 24 acres at Jalalpur Pirwala research farm of Muhammad Nawaz Sharif University of Agriculture, Multan during year 2017. Three selected cotton Bt varieties MNH-992, IUB-2013 and FH-142 expressing Cry1Ac gene sown in the month of May, 2017. Seed was treated with imidachloprid (WS). Safe insecticides i.e., six sprays, were applied against sucking insect pests. There was no Bollworms infestation recorded. PB-ropes impregnated with gossypure were installed at the appearance of 1st square @ 120 dispensers / acre for pink bollworm management. Effectiveness of PB-ropes was monitored by sex pheromone traps baited with gossypure pheromone. Results showed that PB-ropes gave very effective control of pink bollworm throughout the season. In PB- rope treated field moth catches were highly reduced as compared to untreated field. PB rope were remained effective up to 105 days. Higher population of Green lacewing was recorded in the field which played an important role as

predator to control whitefly immatures. This IPM programme should go a long way in managing cotton insect pest complex in a sustainable way.

PREFERENCE OF FRUIT FLY ON DIFFERENT COLORS IN MANGO ORCHARD IN MULTAN, PAKISTAN

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Fruit fly is a major destructive pest of mango and of also different fruits such as guava and vegetables (Cucurbitaceae family). There are total 651 described species while among them 50 have been documented to cause negative impact on economy. Beside attacking in the field, it also infests rotten dropped fruits most likely bananas, vegetables like potatoes and other refrigerated items which serve a source for its infection and also while exporting of fruits or vegetables our quarantine department is not much active or applying safety measures. Present study is designed to access the color preferred by the fruit flies. To conduct, this experiment we used different colored bottles available at local levels in Multan. The colored bottle that we used are Black, Blue, Red, Orange and Yellow. Results revealed that yellow color is the most preferred color followed by orange. Hence, we concluded that as our mango is harvested at yellow stage and in our experiment yellow is the most favored color for fruit fly. Thus, causing heavy damage to our economy.

ECOLOGICAL EFFECTS ON POPULATION OF *BACTROCERA DORSALIS* IN DISTRICT NAUSHAHRO FEROZE SINDH, PAKISTAN

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The mango fruit were affected by some pre-harvesting pest problems like fruit flies *Bactrocera* species and ecological factors. The effects of environmental conditions; such as host plants (living) and weather parameters (non-living) factors on the richness and inhabitants. *Bactrocera dorsalis* in mango orchards were worked out. In district Naushahro feroze *Bactrocera dorsalis* were start emerging during the last week of May. Peak occurrence of fruit fly activity was observed during the mid of July. However, a gradual increase was observed during the beginning of June and slowly gradually decline during the mid of August. Correlation studies between host, pest and weather parameters showed significant positive relationship with maximum and minimum temperature and rainfall, while weak negative correlation with the humidity. Obtainability of peak mango fruits were another crucial reason for affecting population fluctuation.

IMPROVING EFFICACY OF METHYL EUGENOL BAIT AGAINST FRUIT FLY SPECIES IN JUJUBE ORCHARD

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Male annihilation technique (MAT) is used throughout the world to manage fruit fly species. The main content of the trap is Methyl eugenol along with sugar and insecticide. This is species specific and environmentally safe way of controlling the pests. This particular study was design to use different food

additives to improve the efficacy of MAT. The experiment was carried out at the Jujube section, Agriculture Research Institute during the start of jujube season 2017. Methyl eugenol was tested in the presence of different food essences i.e. lemon (T1), strawberry (T2), almond (T3) and orange (T4) essences whereas; methyl eugenol (T5) without any essence was used as control. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. The data collection was started from Jan until the end of April. Two fruit fly species; *Bacterocera zonata* and *B. dorsalis* were identified from the captured individuals. The data revealed that highest mean number of *B. zonata* (334.14 ± 7.86) was collected whereas; the mean number of *B. dorsalis* was 17.25 ± 0.75 . The total mean number of *B. zonata* captured during the study period was 552.46, 351.13, 329.42, 390.21 and 301.66 for T1, T2, T3, T4 and T5, respectively. The highest trapped flies were observed in T1, whereas; the lowest mean number of flies were captured in control trapped. Furthermore, the highest numbers of *B. zonata* were captured in the month of April (334.14 ± 7.86) whereas; lowest population was observed in the month of January (6.95 ± 0.27). The mean number of collected individuals in February and March remain 12.28 ± 0.325 and 29.61 ± 1.040 respectively. The statistical analysis showed that there is high significance difference ($p < 0.001$) between all the treatments. Similarly, the highest mean number of *B. dorsalis* was recorded in April (17.25 ± 0.75) and the minimum density was recorded in January (2.17 ± 0.16). Treatment wise the highest number of *B. dorsalis* was recorded at T1 (46.68) followed by T4 (33.97), T2 (25.68), T3 (25.34) and T5 (17.02). The relation of temperature and wind velocity with *B. zonata* and *B. dorsalis* noted was positively correlated whereas; there was negative relation with humidity. On the basis of above mentioned results that the presence of difference food essences can enhance the capability of methyl eugenol to attract the fruit flies.

EFFICACY OF *MORINGA OLIFERA* AND *TERMINALIA CHEBULA* SEED EXTRACTS AGAINST *TRIBOLIUM CASTANEUM* HERBST

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The efficiency of ethanol extracts of *Moringa oleifera*, *Terminalia chebula* and 1:1 mixture of these extracts in 10mg/L, 30 mg/L and 50 mg/L concentration solutions was studied against *Tribolium castaneum*. Mortality test, contact toxicity test, grain weight loss test and repellency test were carried out. Intermediate level of toxicity was shown by all three extracts. Similarly intermediate effectiveness in preventing grain weight loss was also seen. Most effectiveness in case of preventing the grain damage and toxicity was showed by 50 mg/L concentrations. Concentrations of different extracts studied were directly proportional to the toxicity against *T. castaneum*. It was observed that no relationship was found between toxicity and the type of extract used. Moreover, intermediate level of repellency was showed by all three extracts.

RECOGNITION OF *BEAUVERIA BASSIANA* AS BIO-PESTICIDES IN AGRICULTURE

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Integrated pest management is becoming more and more important tool for the sustainable insect control in ergo ecosystem. During the present study *Beauveria bassiana* was utilize as microbial agent to reduce the pest population of desert locust (*Schistocerca gregaria*) which is big threat to agricultural production because of its ability of swarms to fly rapidly across great distances. Its breeding season is too fast there is 2 to 5 generation per year. History is full of its major and minor plagues which bring negative impact on food security in the region. At present, the chief method of controlling desert locust infestations is use of insecticide which acquires insect directly. But unfortunately, use of this insecticide destroyed the other web-chine system. Present study

recommends that use of bio-pesticide against the locust is effective if it is applicable on solitary phase. This hypothesis let us to use the *Beauveria bassiana* against *Schistocerca gregaria* under laboratory condition. To test the effect of the *Beauveria bassiana* isolates on *S. gregaria*, 02 different formulations i-e (water and oil based) were prepared and individual were treated with this. For this, 10 adult were reared in 5 liter plastic jars while, 10 adult were kept in cage. Thereafter, the treated adult insects were checked for mortality after 1st, 3rd, 5th, 7th, 9th, 11th, 14th and 21st days of incubation. Further, dead insects were transferred to moist sterile 90 mm glass Petri dishes to determine the mycosis rate. The highest mortality rate i-e 85% was obtained at the end of 7th days of incubation treated with oil formulation opposing to this it was 60% on water based medium. During trials it was noticed that in order to upsurge the locust's population in field this bio-pesticide should be commercialized on large scale.

FLUCTUATION IN APHID POPULATION IN DIFFERENT VARIETIES OF WHEAT FROM LARKANA DISTRICT

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Triticum aestivum (wheat) is considered very important food crop in Pakistan including Sindh. This crop is attacked by numbers of insect including aphids, which is responsible for reduction of its yield. Aphid commonly known as plant lice is serious pest of many crops. They damage the crops by sucking its cell sap due to which plant becomes weak and ultimately bears less fruits/seeds. Beside this, it was also noted that aphid throwing honey dew on the leaves of plants, which initiates the development of sooty mould that ultimately affect the photosynthesis process. They also cause much fatal disease. The occurrence of aphid has been started in the month of January and its peak infestation was observed during mid March when wheat crop was fully grown. During the field survey it was noted that aphids were observed first on leaves then they started shifting to the ears when ears started appearing in the last week of February. At present significant large numbers of specimens were collected from the five varieties of wheat i-e TD1, Benazir, Tj83, QS4, NIA Amber. The collected material was sorted out in to two species viz: *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani). During this study it was noted that TD1 was significantly affected by *R. padi* and *S. graminum* as compare to other tested varieties. It was observed that aphid reached peak point in mid February on leaves then in the end of February aphids started shifting from leaves to ears. While, their population start to decline in month of April.

PREVALENCE OF INSECT ON DIFFERENT VARIETIES OF SUGARCANE IN DADU DISTRICT

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Sugarcane plays vital role in the field of Pakistan's agricultural economy, in Pakistan the industrial crop of sugarcane is occupying 1128.10 thousands /ha, yearly production of 63718.52. During the present investigation weekly survey have been carried out in District Dadu and the study area was divided into 08 sectors i-e Payaro Goth, Patt Shareef, Kakar, Phulji, Buriri, Sade Mosani, Makhdum Bilawal and Sita where 04 varieties of sugarcane i-e SPF-234, CPF-237, BL4 and Thatta-10 are growing. During the field inspection significant large number of insects have been seen i-e Stem Borer (*Chilo tumidicostalis*), Internode Borer (*Chilo sacchariphagus indicus*), Shoot Borer (*Chilo infuscatellus*), Root Borer (*Emmalocera depressella*), Black Bug (*Cavelerius excavates*), White Woolly Aphid (*Ceratovacuna lanigera*), Leaf hopper (*Pyrilla perpusilla*) and Thrips (*Fulmekiola serrata*) these pests causes usually damage to quantity, quality and also reduce the sucrose of cane. Mostly *Chilo* species were found in all sectors in the field and damage approximately 10 to 80% of cane and somewhere causing "dead heart". It was also noted that Black bug, Leaf hopper and Thrips cause less damage than *Chilo* species and feed on leaves of cane and White woolly aphid damage even less than above

mentioned species or somewhere minor damage and they found in a few sectors of surveyed areas of the sugarcane crop. Hopefully, this study will be proved guide lines for management authorizes in future.

**STUDY ON DIFFERENCES IN SOME NEW CANOLA CULTIVARS FOR HAVING
RESISTANCE AND SUSCEPTIBILITY FEEDBACK INFECTED WITH
APHID (*L. ERYSIMI* (K.) HEMIPTERA: APHIDIDAE)**

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Assessment of antixenosis free choice test and no choice test was carried out in the randomly selected cultivars of rapeseed against *L. erysimi* (K.). Selection of cultivars was based on our previous findings, categorized into different levels of resistance/susceptibility. The canola aphid observed more enjoying to spent a lot of time for feeding purpose on Abaseen followed by Zahoor while lowest counted preference was recorded on NARC after that Oscar as devoted by the numbers of aphid that sitting on aphids per plant of these cultivars at 12h, 24h and 48h after release/introduction of aphids in a free choice experiment as well as in no choice experiment at different stages (four to six leaves, flowering and pod). NARC exhibited strong antixenosis against canola aphid in no choice experiment. Total ten of genotypes were randomly selected for antixenosis test from the pool of different levels of resistance/susceptibility. In antixenosis test, mean aphid per plant was recorded at four to six leaves stage on NARC followed by Oscar (1.50), (8.56), at flowering stage (1.00), (3.40) and at pod stage (0.90), (2.91) proved to be highly resistant, while Zahoor followed by Abaseen (16.7), (14.0) at four to six leaves stage, at flowering stage (17.6), (15.3) and at pod stage (16.8), (14.7) proved to be highly susceptible in terms of mean number of aphids/plant.

**EGGS EXPOSURE TO LOW TEMPERATURE REASONS DWINDLED VIABILITY, DELAYED
INCUBATION AND IMPEDED BIOLOGY IN GREEN LACEWING
(*CHRYSOPELTA CARNEA* STEPHEN)**

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Green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) is well known as generalist predator. Integrated pest management (IPM) strategies of various crop belongs to its predatory performance. The possibility of storing chrysoperla eggs at low temperature and the effects of storage on viability, emergence and quality of predator and its progeny were test. Eggs were stored up to a month at $4 \pm 1^\circ\text{C}$ in a refrigerator, RH = $65 \pm 5\%$ and in full darkness. Percent hatching, incubation period, larval duration, pupal duration and weight and percent adult emergence were evaluated after every 24 hours. The number of hatched larvae, incubation period and biology of individuals were greatly affected from seven days of cold storage onwards. Hatching were endured only 51.25 %. However, 100 % egg viability were lost after 18th day of cold storage. In incubation period 20.7 % and 41.4 % increase were observed at 7th and 18th day of cold storage respectively. Larval and pupal duration were found longer for the eggs those were stored at low temperature. Larval duration were increased six hours at 7th day. However, this increase expended up to 24 hours at 18th day of cold storage. As concern of pupal duration, big upsurge was found during last days (from 12 days of cold storage onwards), which raised up to 42 hours as compared to control. Hasty reduction were found in pupal weight as cold storage increased and on average it recorded up to two milligrams/pupae. Percent emergence of adults were significantly reduced as cold storage days increased. However, 2-18% reductions were recorded during the studies. No significant effect was found on hatching of F1 and F2 progenies. The results of this study reveal that for more efficient biological control, there is an urgent need to improve the method of storing eggs of *C. carnea*.

INFESTATION OF FRUIT FLY *BACTROCERA* SPECIES (DIPTERA: TEPHRITIDAE) ON VARIOUS FRUIT VARIETIES FROM UPPER SINDH

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Genus *Bactrocera* species (Diptera: Tephritidae) common and most important pest attacks on the different fruit Gauva, Mangoes, citrus fruit lemon, oranges. *Bactrocera* pest is responsible for the low production of yield in Pakistan especially upper Sindh regions are more affected. *Bactrocera* species are considered serious pest of fruit in Sindh, Direct losses in yield 50-80% damage quantity and quality of fruits. Genus *Bactrocera* feed almost-non crop vegetation, unripe or ripe fruits, plant protein, forage Sorghum, grain Sorghum sweet Corn, sugarcane and cassava. During the present study a wide ranging Orchards survey and collected about of fruit flies from the different district and Taluka specially Larkana, Kamber Shahdadkot, Mirokhan & Dokri Bakrana Taluka's and visited Larkana District 1.5 km away from Larkana "Pir Jo Goath" or Village of Akber Rashidi. During the field inspection it was noticed that significant population of *Bactrocera* was seen in field and caused maximum loss to fruit.

ENHANCEMENT OF CHEMOTAXIS EFFECTS OF METHYL EUGENOL AGAINST FRUIT FLY SPECIES IN GUAVA ORCHARD

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Guava is an important fruit of Pakistan and attacked by many insects especially fruit flies which heavily damage this fruit and effect quality and quantity at considerable level. The use of chemicals against the pest is not sufficient for this fruit because maggots damages inside the fruit and also have residual effect to human health. The alternative methods such as male annihilation technique are successfully used against fruit flies in which methyl eugenol is used along with sugar and insecticide. This experiment was designed to use different food additives in methyl eugenol traps to improve the attraction of lure. The experiment was conducted at Guava orchard farm in Agriculture Research Institute (ARI) Tando Jam during guava season 2017. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. The chemical composition of Methyl Eugenol (ME 4ml/wick) (Methyl Eugenol 85% + Sugar 10% + Insecticide 5%) and 4ml/wick different food essences in two different cotton wicks in each treatment i.e. T1 (ME+ lemon essence), T2 (ME + Mango essence), T3 (ME + Vanilla), T4 (ME + Orange essences) and T5 was used as control has only ME solution. Two Tephritidae species *Bactrocera zonata* and *B. dorsalis* were captured. The highest trapped *B. zonata* were observed in T1 Lemon (312.72±4.39), whereas; the minimum number of flies were captured in T5 (225.39±4.47). The results indicate that the maximum numbers of *B. zonata* were captured during the month of April (238.81±1.39) while; lowest population density was observed in the month of January (3.23±0.41). The highest significant difference ($p < 0.001$) recorded between all the treatments during analysis of data. Treatment wise the maximum number of *B. dorsalis* was collected in T1 (30.98±1.94), whereas; minimum number of flies captured in T4 (17.62±1.64). The highest mean number of *B. dorsalis* was observed in April (15.1±0.10) and the lowest population was noted in January (0.33±0.19). Correlation coefficient of temperature and wind velocity with all treatments remained positive whereas; there was negative relation of humidity recorded in all treatments. On the basis of above mentioned results that the presence of different food essences can enhance the capability of methyl eugenol to attract the Tephritid fruit flies.

**REPELLENT EFFICIENCY OF DUSKY COTTON BUG *OXYCARENUS* SPP.
(HEMIPTERA: LYGAEIDAE) UNDER DIFFERENT BOTANICALS**

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Dusky cotton bug has acquired a status of major pest of cotton within the recent years which affect the lint and seed quality. A study was carried out to access the efficiency of different plant extracts that was available locally under the lab conditions of 25±2°C and 70±5% RH in Muhammad Nawaz Shareef University of Agriculture, Multan. Extracts that was tested are as follows Neem (*Azadirachta indica*), Marigold (*Calendula officinalis*), Eucalyptus (*Eucalyptus camaldulensis*), Osmium (*Ocimum tenuiflorum*) and Tobacco (*Nicotiana tabacum*). All the selected plants leaves were shade dried for a period of twenty days and then grinded to make a powder. A solution of each extract was made by using 5g and bioassay was done by the leaf dip method. Data was recorded after 24, 48 and 72 hours. Statistical analysis shows that Neem was the most effective among the others showing the highest repellent efficiency of 56% while Osmium 41% Eucalyptus 40% and Tobacco 30 % respectively.

**SCREENING OF SOME TOMATO (*SOLANUM LYCOPERSICON*)
VARIETIES AGAINST SUCKING INSECT PESTS**

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For screening of tomato varieties against sucking insect pests four tomato varieties were sown; Dominator, Yaki, 1359, 1225, in a Randomized Complete Block Design with three replications at Agricultural Research Institute Tando Jam during Rabi season of 2014 and 2015. The results indicate maximum per leaf whitefly *Bemisia tabaci* population on Dominator further on Yaki, 1359 and 1229 respectively. Jassid *Amrasca biguttata biguttata* population was highest on Yaki, and on 1359, 1229 and Dominator respectively. Aphid *Aphis gossypii* population was recorded highest on 1229, then on Yaki, 1359 and Dominator. Thrips *Thrips tabaci* population was recorded higher on 1359 then on 1229, Yaki and Dominator. Overall Dominator was resistant against jassid, aphid and thrips, but it was susceptible against whitefly, the reason behind are the morphological characteristics of variety Dominator.

**ON POPULATION OF ONION MAGGOTS (*DELIA ANTIQUA*, MEIGEN, 1826) IN ONION CROP
FROM DISTRICT MANSEHRA KHYBER PAKHTUNKHWA, PAKISTAN**

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Present study was carried out during the year 2016-2017 from various fields of onion crop in district Mansehra Pakistan. A total of 182 samples were collected of onion maggots (*Delia antiqua*) by hand picking method. Additionally, field wise population is provided in this paper. This study provides a basic information of this pest from this region and will be beneficial for future studies.

ACROSS GENERATION PLASTIC THERMAL TOLERANCE OF CEREAL APHIDS *RHOPALOSIPHUM PADI* LINNAEUS AND *SITOBION AVENAE* FABRICIUS

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Ecological consequences of global warming and climate change are being evident all over the world. High temperature events influence the demographic parameters and population performances of animals including insects. Exposure to heat stress may exert significant impact on the thermal tolerance of insects including herbivore pests. This study aimed to determine the thermal tolerance indices *i.e.* basal and acclimated critical thermal maxima (CT_{max}) and chronic thermal tolerance for *Rhopalosiphum padi* and *Sitobion avenae*, the two most important and prevalent cereal aphids species all over the world. Aphid clones were collected from field randomly and were reared in laboratory at 20±1 °C and 50-65 % RH with 16L: 8D hr photoperiod. Five-day old female aphids from parental, F1, F2 and F3 generations were either exposed directly to chronic temperature of 31 °C until their death or their basal and acclimated (3h at 34 °C) CT_{max} indices were determined using a programmed glycol bath. Results revealed that thermal thresholds (both basal and acclimated CT_{max} indices) gradually increased from parental to F3 generation and increased as well from 2nd day offsprings to 4th day offsprings. Across generations, basal and acclimated CT_{max} values ranged respectively from 36.52 °C for parental generation to 37.10 °C for F3 generation, and from 37.44 °C for F1 generation to 37.83 °C for F3 generation for *Sitobion avenae*, and for *Rhopalosiphum padi*, ranged from 37.28 °C of F1 generation to 37.58 °C of F3 generation, and from 38.11 °C of F1 generation to 38.53 °C of F3 generation respectively. *R. padi* thermal threshold values, on average, were approx. 0.77 °C higher than those of *S. avenae*. For both species, higher chronic tolerance (to 31 °C) was exhibited by parental or F1 generation than later generations and by 2nd day offsprings than 3rd or 4th day offsprings. These differential thermal indices and population performances of both aphid species could be due to the impact of chronic and acclimated temperatures on the density and diversity of obligatory and facultative bacterial endosymbionts these species harbor within their bodies.

EFFICACY OF DIFFERENT PROTECTIVE AND CURATIVE FUNGICIDES AGAINST CITRUS UNDER AGRO-ECOLOGICAL CONDITIONS OF SARGODHA

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Plant pathogenic fungus *diaporthe citri* is the main casual organism of citrus melanose disease on different citrus varieties. Fungus cannot effect the pulp of fruit whereas it causes severe rind blemishes on fruit and raised black lesions surrounded by yellow halos on leaves. This disease has quarantine importance as well decrease the fruit export price and increase the fruit rejection ratio in international market. A trial was conducted at the farmer's field in district Sargodha, Pakistan under irrigated condition during 2017. Research was started from last week of April 2017 after 80% of petal fall was completed. Five different fungicides were applied as protective and curative viz: Nativo® (Tebuconazole+ Trifloxystrobin) @ 0.6g/L, Amistar (Azoxystrobin + Difenoconazole) @ 1ml /Liter, Cabrio top® (Pyraclostrobin+ Metiram) @ 2g/Liter, Cobox (Copper oxychloride)@ 4g/liter and Kocide®(Copper hydroxide) 2g/liter to check their efficacy against *diaporthe citri*. All fungicides gave satisfactory results after 15, 30 and 45 days, Cabrio Top® had good results after 15 days whereas Amistar top was statistically significant in reducing melanose symptoms up to 45 days and could be recommended as preventive and curative treatment for the management of citrus melanose.

DAMAGE OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: CHRYSOMELIDAE) ON COWPEA SEEDS DURING STORAGE

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Cowpea (*Vigna unguiculata* L. Walpers, Fabaceae), is an important edible legume crop in many parts of the World especially in tropical and subtropical regions. It is used as human food due to its high protein content and also as live stock feed to make silage and hay. The cowpea weevil *Callosobruchus maculatus* (F.) is a cosmopolitan field to store pest ranked as the principal post harvest pest of cowpea grains in Sindh Pakistan. A laboratory study was conducted to investigate the damage of *Callosobruchus maculatus* on cowpea seeds. The cowpea variety black eyed beans was used in the study. Five pairs of *Callosobruchus maculatus* was released on 500g of cowpea. Findings of this experiment revealed that cowpea seeds were the most sensitive pulse grains against the *Callosobruchus maculatus* (F.) infestation, because 93.2% seeds were found damage from October to November 2017 during the storage period of eight weeks.

SUB-LETHAL EFFECTS OF SPINOSAD ON THE BIOLOGY OF RED COTTON BUG (*DYSDERCUS KOENIGII*) UNDER LABORATORY CONDITIONS

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Red cotton bug, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae) is an important pest of cotton worldwide. It causes damage to the crop by feeding on developing cotton bolls, and ripened cotton seeds. In the current study, effect of spinosad on the biology of this pest was studied under laboratory conditions. Duration of nymphal stages, nymphal weight, nymphal survival, numbers of adult and numbers of eggs laid were studied in spinosad exposed and un-exposed individuals. There was significant difference observed between the spinosad treated and untreated individuals in terms of above parameters. Spinosad treated individuals had longer nymphal duration (4-7 days) but less weight (19.65-44.75 mg) as compared to untreated individuals. The egg laying capacity was also significantly reduced (0-55 eggs/female) in spinosad treated individuals as compared to individuals in control (22-75 eggs/female). The survival of adults was also minimum in spinosad exposed individuals (68%) compared to untreated individuals (100%). The results suggested spinosad not only kills cotton stainer but also affect its biology. The findings will be helpful in designing pest management strategy for this destructive pest.

A REVIEW ON COMPREHENSIVE STUDY OF MANGO FRUIT FLY AND THEIR EFFECTIVE MANAGEMENT STRATEGIES

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Mango (*Mangifera indica*), the king of fruit is grown in large area typically opposed by two or three crucial pests, numerous secondary pests and majority of irregular pests. In the world Fruit flies (Diptera:Tephritidae) are the greatest attentively chief pest species of fruits and fleshy vegetables throughout tropical and sub-tropical zones. Fruit fly infestation is customarily originate in mango producing country of the world and the producers suffering significant conventional and unintentional commercial losses consequential from fruit fly destruction. Mango fruit is attacked by reported 48 species. Genera *Bactrocera* (30 species),

Anastrepha (8 species), *Ceratitis* (7 species), *Dirioxa* (2 species) and *Toxotrypana* (1 species). In July and August 30% of mango fruits is attacked by *B. dorsalis* and 35% of mango fruit is attacked by *B. zonata* in Central Punjab. To control of these species eradication programs have been established in various portions of world. The arrangement of control approaches contains hygiene, cultural controls, insecticide sprays to foliage and soil, bait-sprays, male annihilation techniques (MAT), pheromones, proclamations of sterilized flies and parasitoids. Throughout the twenty first period there has been a development to interchange away from control with organophosphate insecticides (e.g., Malathion, Diazinon, and Naled) and towards reduced threat insecticide treatments. In tropical and sub-tropical areas advance technologies are established to reduce the threat of fruit fly to control and explore Integrated Pest Management (IPM) Programs that take part numerous mechanisms to accomplish the fruit fly management.

IDENTIFICATION, HOST PLANTS, COLOUR AND VARIETAL PREFERENCE OF TOMATO LEAF MINER FOUND IN MULTAN (PAKISTAN)

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Tomato (*Lycopersicon esculentum*) is one of the most important vegetables consumed worldwide with annual global production of approximately 159 million tons. These are consumed as fresh, used in cooked vegetables, the processing industry as well which make tomatoes the world's leading processed vegetable. Tomato leaf miner is an emerging threat to tomato crop in Pakistan especially on hybrid varieties of tomato. Inadequate information is available so far in Pakistan. A leaf miner is an immature of an insect that lives inside and consume the leaf tissues of plants. Usually, leaf miners belong to four orders of class insecta, Lepidoptera (moths), Symphyta (wasps), Diptera (Flies) and Coleoptera (beetles). Present study was carried out at MNS University of Agriculture, Multan and surrounding farmer fields. Samples of infested leaves of tomato and other plants were collected from farmer fields and identified in laboratory under microscope. We identify that leaf miner attacking tomato plants and other crops are minute flies which belong to order Diptera, family Agromyzidae and Genus *Liriomyza*. We recovered these flies from almost twenty different host plants belonging to field crops, vegetables and ornamental plants etc. Sticky traps of two colours blue and yellow were evaluated in farmer fields. Leaf miner flies were most attracted towards yellow colour. Varietal preference was also evaluated on four varieties of tomato i.e. Baby red, Super, ICI-1205 and Sehar in field trials. Varietal preference was evaluated on the basis of percent infestation of tomato plants. Our results revealed that all four varieties of tomato are susceptible to leaf miner attack. Among four varieties tested Baby red showed highest infestation (55.88%) as compared to others i.e., Super (47.84%), ICI-1205 (44.01%) and Sehar (41.84 %).

EFFECT OF INSECTICIDE, ACEPHATE ON BEHAVIOR OF *OXYOPES JAVANUS*

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Sub lethal doses of insecticides are known to alter the behavior of insect pests' predators. Our objective was to determine that whether sub lethal exposure (LC₅₀) of excessively used organophosphate insecticide, acephate could affect the consistency of individual behavior, in a foliage spider *Oxyopes javanus* (Thorell, 1887) most commonly occurring in agro ecosystems of Pujab, Pakistan. The insecticide was applied on the body of adult spiders by immersion method. Change in climbing, courtship, locomotion and avoidness behavior of untreated and treated spiders was recorded. All results were compared statistically. It was observed that Acephate affected the climbing activity, Open field activity, Avoidance, locomotion and Courtship behavior of *Oxyopes javanus*. Present study showed that acephate is highly toxic insecticide for *Oxyopes javanus*, and even

its sub lethal concentrations have potential to disrupt the normal behavior. So the use of acephate should minimize in agroecosystems. And only those insecticides should be used in the fields which are less toxic, targeted to control pests and are not harmful to predators. It is suggested to investigate other commonly and excessively used insecticides for their potential to alter the behavior of insect pests' predators.

SCREENING OF DIFFERENT CHICKPEA (*CICER ARITENUM* L.) VARIETIES AGAINST POD BORER (*HELICOVERPA ARMIGERA* H.) UNDER NON SPRAYED CONDITIONS

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Chickpea (*Cicer aritenum* L.) is major consuming food in Pakistan. It can be grown easily as its production is very inexpensive and can be done even in unfavourable environmental conditions. The only threat to chickpea is the attack of pod borer (*Helicoverpa armigera* H.), which causes significant losses to this crop. Due to the developing trend of insecticide resistance in pod borer, it is becoming difficult to control it. In this experiment, ten chickpea varieties (BRC-61, BRC-388, BRC-390, BRC-402, BRC-403, BRC-404, BRC-405, BRC-427, CHAKWAL-11 and PB-2008) were sown in the farm area of Regional Agricultural Research Institute (RARI), in Randomized Complete Block Design (RCBD) with three replications. Screening of these varieties was done against the infestation of pod borer under non-sprayed conditions. Within the time frame of germination to harvesting, counting of pod borer population was done in one meter length of every row. The analysis of data was done statistically by using Analysis of Variance (ANOVA) and the means were separated by Least Significance Difference (LSD) at 5% probability level. It was found that BRC-405 and BRC-402 showed highly resistant response against pod borer with least damage percentage (7.577%, 9.352%), respectively as compared to all other tested varieties. CHAKWAL-11 showed high level of pod borer damage percentage (21.242%) than all other varieties.

EXPLORATION OF INSECTICIDAL BIOACTIVITIES OF ESSENTIAL OILS OF INDIGENOUS MEDICINAL HERBS AGAINST *CALLOSOBRUCHUS CHINENSIS* (FAB)

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Callosobruchus chinensis is the important pest of stored grains which cause damage to grains. The study was carried out to evaluate the insecticidal potential of weeds collected from different areas of Bahawalpur. Weed essential oils were used to check the mortality and weight loss caused by *C. chinensis* in *V. radiate*. Weeds such as (Lehli, Lehu, Bathu, Krund, Sinji) were collected from different areas of Bahawalpur. For the evaluation of toxicity, filter paper was treated with essential oils and placed in the petri dishes. 2%, 4% and 6% concentration of essential oils were used to check the mortality against *C. chinensis*. The recorded data showed that 100% mortality was shown by Lehu weed while minimum mortality (13.2, 21.66 and 28.33) was shown by lehli weed against *C. chinensis*. For estimation of weight loss twenty insects of *C. chinensis* were released in each jar containing weighed amount (20gm) of *Vigna radiate* seeds. The seeds of *V. radiate* were treated with three concentrations (2, 4 and 6%) of essential oils following three replications. Weight loss was observed after 15 and 30 days following counting and weighing method. The application of essential oils of *Cirsium arvense* with respect to other weed oils exhibited weight loss (0.44%, 0.44%, 0.00) caused by *C. chinensis* in mung bean seeds at 2, 4 and 6% concentrations. The application of essential oils of other weeds (lehli, krund, bathu, sinji) on mung bean seeds exhibited weight loss lehli (2.50%, 2.58%, 4.05) krund (4.83%, 5.16%, 4.40%) bathu (3.66%, 4.33%, 2.75%) sinji (3.46%, 4.00%, 4.33%) caused by *C. chinensis* at 2, 4 and 6% concentrations respectively. The results concluded that *C. arvense* showed greater mortality in the population of *C. chinensis* with respect to other weeds.

**POPULATION DENSITIES OF *TRYBLIOGRAPHA DACI* AND
DIACHASMIMORPHA LONGICAUDATA ON DIFFERENT CUCURBIT CROPS**

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Natural enemies plays vital role for the management of insect pests. However, their population fluctuates drastically in the field due to several reasons. In connection to this, a field experiment was undertaken to carry out for the surveillance of natural enemies of cucurbit fruit fly on bitter gourd *Momordica charantia*, bottle gourd *Lagenaria siceraria* and Indian squash *Praecitrullus fistulosus* from Mirpur Khas and Hyderabad, Sindh, Pakistan. Infested fruits of *M. charantia*, *L. siceraria* and *P. fistulosus* were collected, weighed and kept in fruit fly laboratory for recording the parasitoids of cucurbit flies at monthly intervals. The parasitoids were identified, counted and recorded. Results depicted that two larval parasitoids viz *Diachasmimorpha longicaudata* and *Trybliographa daci* were recorded from three vegetable crops. Higher numbers of *T. daci* were recorded from all the tested field infested fruits as compared to *D. longicaudata* population densities. However, only single parasitoid *T. daci* was recorded from Indian squash throughout course of experiment. These results would be helpful for the management of cucurbit vegetables.

**STUDY ON INSECTICIDAL ACTIVITY OF PLANT ESSENTIAL OILS AGAINST KHAPRA
BEETLE, *TROGODERMA GRANARIUM* E. (COLEOPTERA: DERMESTIDAE)**

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Trogoderma granarium E. is the most destructive pest of stored grain products. Application of chemicals use to control the infestation but it has several harmful effects on the quality of grains, human health and environment. The objective of study to identify the toxicity of methanolic plant essential oils and optimum concentration on *Trogoderma granarium*. Five essential oils *Citrus limon*, *Azerchita indica*, *Ficus benjamina*, *Ziziphus mauritiana* and *Syzygium cumini* were tested against *Trogoderma granarium* at concentrations 25%, 50%, 75% and 100% with time interval 12, 24 and 36 hours. The experiment was laid out in completely randomized design (CRD) with the three replication for each concentration. The results showed that higher concentration of essential oils resulted in maximum repellency for maximum exposure period as compared to minimum oil concentrations at minimum exposure period. Furthermore, it is concluded that *Azerchita indica* and *Ziziphus mauritiana* proved more toxic whereas *Ficus benjamina* and *Syzygium cumini* were showed least toxic. The Citrus limon showed very low toxic effective against *Trogoderma granarium* at all the concentrations.

**MOLECULAR ANALYSIS OF THE VENOM OF PARASITIC HYMENOPTERA: AN INNOVATIVE
APPROACH FOR THE DEVELOPMENT OF NOVEL PLANT PROTECTION
STRATEGIES FOR SUSTAINABLE INSECT CONTROL**

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Venom is one of the important component of the reproductive secretion of insect parasitoids is a rich source of novel bioactive genes and peptides with potential insecticide activities which may be exploited for the development of natural plant protection strategies in agriculture. Development of biologically based pest

management strategies which are ecologically sound, economical, efficacious, improve food safety and that are acceptable to the public are prerequisites to meet the challenges of WTO for pest management and phytosanitary measures. A little information is available on the factors of parasitoid origin able to modulate host physiology. The venom is one of the key factors involved in host regulation process by parasitic Hymenoptera. It is a complex mixture of proteinaceous compounds injected by the ovipositing females of the parasitoid in their host insects. Molecular analysis (SDS-PAGE) of the venom of the wasp was performed with the main objective of having a profile of total proteins occurring in the venom blend of insect parasitoid *Aenasius arizonensis* (Girault) (= *Aenasius bambawalei* Hayat) (Hymenoptera, Encyrtidae) a newly discovered solitary endoparasitoid of the cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera, Pseudococcidae), is a potential insect control tool. In continuation of our previous work, a transcriptional profile of the venom gland tissues of the female wasp was also developed for fishing out bioactive genes/pptides with potential insecticidal activity by utilizing high throughput RNA sequencing approaches followed by *de novo* assemblies which yielded a number of contigs with significant BLAST homologies in NR database which may be exploited as candidate genes for the development of insect control strategies through integrated approaches of molecular and functional studies

CHRONOLOGICAL ASSOCIATION OF FOLIAGE INSECTS AMONG DIFFERENT CITRUS ORCHARDS

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Overall 8625 specimens were recorded from three orchard fields as a whole and least population was recorded from Fruiter orchard fields 33.19 % (N = 2863) then population was recorded from Kinnow orchard fields 33.36 % (N = 2877) and maximum population was recorded from Mosambi orchard fields 33.45 % (N = 2885). Taxa composition also recorded from Kinnow orchard fields, entire population was recorded pertaining to 6 orders, 37 families, 54 genera and 58 species. From Fruiter orchard fields, population was recorded pertaining to 6 orders, 34 families, 49 genera and 56 species. Wherein from Mosambi orchard fields, population of foliage insect's fauna was recorded pertaining to 6 orders, 33 families, 48 genera and 52 species. From Mosambi orchard fields, maximum species abundance was recorded during 9th sampling (14 species) at 32°C temperature and 34% humidity; in case of Fruiter, maximum species abundance was recorded during 11th sampling (14 species) at 32°C temperature and 56 % humidity and in case of Kinnow maximum was recorded during 5th and 9th sampling (12 species) at 30°C temperature and 16% humidity. From Mosambi orchard fields, it was accessed that *Maconellicoccus hirsutus* (Pseudococcidae) was existing with maximum relative abundance of 89.25% (N = 2575), in Fruiter orchard fields, *Maconellicoccus hirsutus* (Pseudococcidae) was existing with relative abundance of 88.44% (N = 2532) and in Kinnow orchard fields, *Maconellicoccus hirsutus* (Pseudococcidae) was existing with maximum relative abundance of 90.30% (N = 2598). In Mosambi orchard fields, maximum relative abundance was recorded for order Hemiptera (Bugs) 90.57 % (N = 2613). In Fruiter orchard fields, maximum relative abundance was recorded for order Hemiptera (Bugs) 90.71 % (N = 2597) and in Kinnow orchard fields, maximum relative abundance was recorded for order Hemiptera (Bugs) 91.87 % (N = 2643). Diversity (H') was recorded highest (2.7213) from Mosambi orchard fields as compared to Fruiter (2.7158) and Kinnow (2.7158) orchard fields. Richness (R) was recorded from Mosambi (14.5860), Fruiter (15.5968) and maximum (15.7855) from Kinnow. Analysis of variance (ANOVA) among Kinnow, Fruiter and Mosambi orchard fields showed non-significant results (F = 0.00; P = 0.1000). Linear Regression confirmed that structural community as well as taxa composition were differing significantly among orchard fields, Kinnow vs. Mosambi (F = 19710.79; P ≤ 0.0000) Kinnow vs. Fruiter (F = 4340.04; P ≤ 0.001) and Mosambi vs. Fruiter (F = 2051.75; P ≤ 0.0000).

EVALUATION OF PESTS VS PREDATORS GUILDS IN CITRUS ORCHARDS

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The present research work was conducted to evaluate "The pest vs predators' guilds in citrus orchards" under the diverse ecological conditions of Faisalabad (Punjab), Pakistan through the session January through December 2016. Overall 381 specimens were documented different no. of population was familiarized from field but variably, maximum population was recorded for pests 77.95% (N = 297) and least population was recorded for predators 22.05% (N = 84). Taxa arrangement also recognized meaningfully diverse from mutually pests and predators e.g. for pest's complete populace was predictable relating to 6 species, 34 genera, 29 families and 7 orders. Whereas for predators, inhabitants were familiarized refer to 5 orders, 16 families, 26 genera and 32 species. For pests, least abundance was known during 9th sampling 3 species at 31°C and 76% moisture. For pests, least relative abundance was documented for genus *Musca* 3.70% (N = 11), *Lucilia* 4.04% (N = 12), followed by *Bactrocera* 4.71% (N = 14), and extreme for genus *Diaphorina* 67.68% (N = 201). For predator, relative abundance was recorded *Lasius* 8.33% (N = 7), *Coccinella* 9.52% (8), followed by *Cheiracanthium* 19.05% (N = 16) and highest for genus *Camponotus* 20.24% (N = 17). Out of 45 noted families, 16 families were documented as pests. Comparative richness was retrieved of family Eutichuridae 19.05% (N = 16), and supreme for family Formicidae 33.33% (N = 28). In case of predator, 24 families were represented as predator, and qualified abundance was by Muscidae 3.70% (N = 11), followed by Tephritidae and Calliphoridae 5.05% (N = 15) and maximum for family Psyllidae 67.68% (N = 201). ANOVA between individually (pests and predators) exhibited non-important consequences (F=1.11; P=0.3172). t-test registered that in general forte. (t-value= 1.19; P-value = 0.2888).

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PROPAGATION OF ORDER DIPTERA AND COLEOPTERA AROUND THE BIOLOGICAL TIME-SCALE AMONG CITRUS ORCHARDS

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From both fields, overall 673 specimens were captured but unpredictably, highest population was recorded from Restricted fields 53.05% (N=357) and slightest population was recorded from non-restricted fields 46.95% (N=316). Taxa composition also recorded significantly different from two fields e.g. from Restricted field, entire population was recorded pertaining to 31 species, 22 genera, 13 families, and 2 orders. In case of non-restricted fields, foliage insect faunapopulation was recorded pertaining to 32 species, 22 genera, 15 families and 2 orders. In Restricted field, highest biomass was recorded during 12th sampling (1.44 ± 0.52) at 59% humidity and 23°C temperature, and least insect biomass was recorded during 5th sampling (0.8 ± 0.07) at 22% humidity and 40°C temperature. Where Non-restricted field, utmost biomass (1.23 ± 0.38) was recorded during 7th sampling at 26% humidity and 36°C, and slightest biomass (0.6 ± 0.06) was recorded during 5th sampling at 20% humidity and 38°C temperature. From restricted fields, *Drosophila melanogaster* (Drosophilidae) was recorded as an astonishing species with greatest abundance of 18.49% (N= 66), and from non-restricted fields, it was accessed that *Musca domestica* (Muscidae), was recorded as a remarkably contributing specie with greatest abundance of 9.49% (N= 30). From total of 15 recorded families, 13 families were calculated from Restricted field. Relative richness was recorded surprising for family Drosophilidae 22.

41% (N = 80), followed by Coccinellidae 19.89% (N= 71), Calliphoridae 14.57 % (N = 52), Ulidiidae 10.92 % (N = 39), Tephritidae 7.00% (N = 25), Muscidae 6.44% (N = 23), Sepsidae 5.88% (N = 21), Nitidulidae 3.92% (N = 14), Sarcophagidae 3.36 % (N = 12). From total of 15 recorded families, 15 families were calculated in non-restricted fields. Qualified richness was recorded for family Ulidiidae 15.51 % (N = 49), followed by Drosophilidae 15.19% (N = 48), Muscidae and Sepsidae 10.76% (N = 34), Nitidulidae 9.18% (N = 29), Tephritidae 8.54% (N = 27), Coccinellidae 7.28% (N= 23), Calliphoridae 6.96 % (N = 22), Syrphidae 5.38% (N = 17). In case of Restricted fields, from total of 2 specific orders, insects of both orders were found, and comparative wealth was recorded amazing for order Diptera (flies) 76.19% (N=272) and for Coleoptera (beetles) 23.81% (N = 85). In Non-restricted fields, insects of all the specific orders (2 orders) were found and comparative abundance was recorded astonishing for order Diptera (flies) 82.28% (N=260) and for Coleoptera (beetles) 17.72% (N = 56). T-test showed that, Foliage insect's fauna was differing considerably in restricted field, (t-value= -2.41; P-value= 0.2502). It was confirmed by Linear Regression that taxa composition as well as structural community were differing significantly among both fields, Restricted vs. Non-restricted (F= 11.55; P ≤ 0.0048).

POPULATION DYNAMIC OF MANGO FRUIT FLY IN TRAPS

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Scientific name of fruit fly is *Drosophila melanogaster* belongs to family (Tephritidae Diptera). Fruit fly was major pest of horticultural crops and fruits including mango, citrus, papaya, banana and cucurbits causing damages and economic losses to fruits and vegetables crops. Fruit fly attack fruits at different stages of maturity but damage is more obvious at maturity. This experiment was conducted in district Multan. The orchard was situated at Moza Buch Mubarak, Nawab Pur road Multan. Following material was used for this experiment. Fruit fly pheromone traps, Cotton, Malathion and permanent marker. Two pieces of cotton were dropped with 10 drops of Methyl Eugenol and one drop of Malathion. These two pieces of soaked cotton were placed in two pheromone traps respectively. The traps were hanged in a line with a distance of 2 meters. We noted data on daily basis. Weather fluctuations (temperature, humidity, daylight) was also checked. These traps were marked with a permanent marker with date. The specie and gender attraction was also monitored. We continuously took the data for one month. We observed that male catching ratio was higher in Methyl Eugenol Traps as compared to Melathion.

REPELLENT EFFICIENCY OF DUSKY COTTON BUG OXYCARENUS SPP. (HEMIPTERA: LYGAEIDAE) UNDER DIFFERENT BOTANICALS

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Dusky cotton bug has acquired a status of major pest of cotton within the recent years which affect the lint and seed quality. A study was carried out to access the efficiency of different plant extracts that was available locally under the lab conditions of 25±2°C and 70±5% RH in Muhammad Nawaz Shareef University of Agriculture, Multan. Extracts that was tested are as follows Neem (*Azadirachta indica*), Marigold (*Calendula officinalis*), Eucalyptus (*Eucalyptus camaldulensis*), Osmium (*Ocimum tenuiflorum*) and Tobacco (*Nicotiana tabacum*). All the selected plants leaves were shade dried for a period of twenty days and then grinded to make a powder. A solution of each extract was made by using 5g and Statistical analysis shows that Neem was the most effective among the others showing the highest repellent efficiency of 30.33 while Eucalyptus 14.33, Osmium 17.66 and Tobacco 21% respectively.

HOST PLANT RANGES OF *MYZUSPERSICAE* AND *APHISFABAE* FROM JACOBABAD DISTRICT

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Aphids are injurious pest of different crops in Pakistan. Aphids not only cause of reducing crops fields but it's also becoming source of many disease Just 92 species of aphids are only present in Pakistan. An extensive survey was conducted during January till May 2017 to study the occurrence abundance of myzus persicae and Aphis fabae (Homoptera: Aphididae) on different host plants in agricultural field around Jacobabad District. During the field investigation for this survey Jacobabad and its Talukas Gharikha and Thul were selected. Four Localities of Jacobabad Gharisabhayo, Dasti, Ali pur and Rind wahi were observed. four localities of Thul Amirabad, Taj Dero, Sultanpur and Jafferabad were visited randomly while three localities from Ghari Kairo Allahabad, Mirpur Burro Rahimabad were under our observation. Total Eleven Localities were observed in whole Jacobabad District. We collected infested plants from different fields. Infested plants were brought into insectary of Zoology Department at University of Sindh Jamshoro. Collected aphids were preserved into glycerin. Infested plants were observed under binocular microscope. Taxonomical keys were used for identification of aphids. As a result, it was observed that two species of aphids *myzuspersicae* and *Aphisfabae* were attacking many host plants. These both species are polyphagous in Nature mean depending on multiple plant or more than one host plant. It was observed that *myzuspersicae* was the most common Aphid's specie attacking about five plant species 1- Brassica 2- cucumber 3- Water Melon 4- Potato 5- Okra while *Aphisfabae* become second most common aphid's species attacking about four Host plants species and those host plants were 1- Lucerne 2- Bean 3- Potato 4- Tomato. Brassica planted infested a lot as compare to other host plants by *myzus persicae* while *Aphisfabae* attacked Lucerne plant more than others host plants. Total 18,899 specimens were collected from both Species but *myzuspersicae* remain high in infestation rate with total 12,339 specimens than *Aphisfabae* which had 6560 specimens as we mention above *myzus persicae* attacking five host plants so Brassica plant infested higher than other four plant 1-Brassica have 5548 specimens 2- cucumber infested by 5249 specimens 3-Water melon attacked by 879 specimens 4- Potato damaged by 458 specimens while 5- Okra remain least attacked with 205 specimens . *Aphis fabae* was damaging by four host plants out of those four Plants 1- Lucerne damaged higher with 4793 specimens while 2- Beans infested by 889 specimens and Potato attacked by 776 specimens while least infestation observed on Tomato with 302 specimens. It was observed that brassica infested a lot brassica plant is count in oilseed plant and this plant very helpful in managing Pakistan economy level by its oil but our finding telling us that this plant infesting a lot by *myzus persicae* while *Aphis fabae* damaging excess by Lucerne plant Lucerne plant count in fodder crop our finding telling us that Lucerne Attacked a lot because its Present throughout year in our study area at Jacobabad that's why its infested a lot by *Aphis fabae* Lucerne also called Alfa Alfa and fodder crop .

MANAGEMENT OF FRUIT FLY BY USING BAGS TECHNIQUE

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Scientific name of fruit fly is *Drosophila melanogaster* belongs family (Tephritidae Diptera). Fruit fly was major pest of horticultural crops and fruits including mango, citrus, papaya, banana and cucurbits causing damages and economic losses to fruits and vegetables crops. Fruit fly attack fruits at different stages of maturity but damage is more obvious at maturity stages. Fruit fly also caused a serious problem in Pakistan specially in mango. As fruit fly is polyphagous, it causes serious damages to the fruits & vegetables. In this technique, different types of bags were used, Chinese bag, Korean bag and American bags and locally made bag was used in mango orchard. This experiment was conducted at Multan near Nawab pur. Firstly divide orchard into three parts. Once is susceptible part, second part is unsuitable varieties and 3rd one was used as control. During this experiment we observed that the Fruits enclosed in bags were healthy un dropped and undamaged. There

was some color shade difference in fruits of different bags. Fruits were in American bags light yellowish and small as compared to fruits caged in Korean and Chinese bags. If we compare the fruits enclosed in Korean And Chinese bags, the result of fruits enclosed in Korean bags were better.

**OCCURRENCE OF *SPODOPTERA LITURA* (LEPIDOPTERA: NOCTUIDAE) IN
CAULIFLOWER FIELDS OF MIRPURKHAS, SINDH**

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Vegetables are the important source of economy and human diet so consumed at large scale. In Pakistan vegetables are cultivated at large scales about one million hector area under vegetable cultivation with total production about 10.00 million tons each year. Beside it vegetables are attacked by variety of insect pest, which caused serious damage and lead reduction of yield *Spodoptera litura* (Lepidoptera: Noctuidae) is the most serious pest of vegetables, causing yield loss ranging from 31% to 100%. It attacks more than 40 plant families. Present study was carried out from September to November 2017 in cauliflower, chilies, and onion and tomatoes fields of district Mirpurkhas. During present study prevalence of *Spodoptera litura* was recorded on all four vegetables but maximum infestation recorded in cauliflower. Infestation of army was observed above ETL in the month of October in cauliflower field. Present study revealed that severe infestation of this pest may demand wide use of insecticides to save the infested crops, which ultimately cause of air and water pollution.

**RESISTANCE OF RICE VARIETIES AGAINST RED FLOUR BEETLE *TRIBOLIUM CASTANEUM*
(COLEOPTERA: TENEBRIONIDAE)**

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Tribolium castaneum H. is the major store grain pest of rice, both larvae and adults feed on rice grains. It makes the rice quality disagreeable and make it unfit for human consumption. Present study was carried out from March 2016- December 2016 on two commercial varieties of stored rice Irri-09, and Irri-6. Adult and larval stages of red flour beetle *Tribolium castaneum* were collected from infested stored rice grains from market and reared in laboratory. 25 adults of red flour beetles were introduced in 100 grams of each variety of rice. The consumption was observed on both varieties. The consumption of red flour beetle on rice varieties were recorded significantly different ($P > 0.05$). The maximum consumption 75.23 grams was recorded on Irri-6 while on Irri-09 it was recorded 44 grams respectively.

**PREDATOR-PREY RELATIONSHIP OF AN APHIDOPHAGOUS HOVER FLY, *EPISYRPHUS*
BALTEATUS (DE-GEER) (DIPTERA: SYRPHIDAE, SYRPHINAE) WITH MUSTARD APHID,
LIPAPHIS ERYSIMI (KALT) (HOMOPTERA: APHIDIDAE) ON *BRASSICA RAPA*
IN SOUTHERN PUNJAB, PAKISTAN**

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As recent investigation has confirmed that the larvae of most of the Syrphid flies specially Syrphinae are predator over many phytophagous insects, keeping in view above investigation the current investigation is carried out from southern Punjab Pakistan, which aimed at finding out the relationship of the one of the most common species of aphidophagous hover flies namely *Episyrphus balteatus* with the mustard aphids

(*Lypaphiserysimi*) on *brassica*. Mustard aphid is most destructive insect pest belonging to the category of polyphagous insects which attack the inflorescences, leaves and stems of plants results in total discoloration and destruction of that part of plant body. The present study was conducted from southern Punjab Pakistan from January to April 2017. In current investigation 312 specimens of *Episyrphus balteatus* along with 12 larvae were collected from brassica crop with the help of sweeping net, malaise trap and hand picking. Through investigation it was found that the co relation of predator was strongly positive with the density of it's pray aphids, while predator displayed negative correlation with the, humidity, temperature, wind velocity and rain fall in the studied ecosystem. The present findings of aphidophagous hover flie's potential and biotic interactions with reference to the *Episyrphus balteatus* relationship to prey feeding ability and seasonal abundance of Predators in relation to the density of the prey *L. erysimi* will be proved supportive to exploit these natural predator against phytophagous like mustard aphids.

BIOLOGY AND INFESTATION OF *ZAPRIONUS INDIANUS* ON BER FRUIT IN HYDERABAD DISTRICT

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Fruit fly *Zaprionus indianus* is polyphagous pest of many fruits throughout the world and cause severe infestation. In Pakistan its infestation observed on ber fruit and causing low yield and poor quality of fruits. Present study of Biology and Infestation of *Zaprionus indianus* on ber fruit was carried out in laboratory. *Zaprionus indianus* first time reported from study locality especially from ber fruit. During present study three successful generations of *Zaprionus indianus* were observed. The larval developmental time of *Zaprionus indianus* was recorded 4-16 days and Pupal time was 3-6 days. Adult life span was recorded 4-18 days. Female starts laying eggs after 2-4 days of mating, egg fecundity was recorded 28%. The whole life cycle of *Zaprionus indianus* completed in 16-35 days Life cycle of *Zaprionus indianus* successfully completed in ber fruit in laboratory. This paper is from the higher education commision, Islamabad funded project no: 20-3838/NRPU/R&D/HEC/14.

OCCURRENCE OF APHID SPECIES (HOMOPTERA: APHIDIDAE) IN THATTA SINDH, PAKISTAN.

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Aphids belong to order-Homoptera containing 4000 described species all over the world. Some of these are serious pests of crucifer crops, vegetables and fruit trees. Aphids are soft bodied, louse like insects, readily distinguished by rounded form and paired siphunculus on the abdomen. Aphids are obligatory parasites of plants. Their notoriety, polyphagous habit and cosmopolitan distribution have gained worldwide importance. We have surveyed different fields of Thatta and collect the aphids from various parts of plants, grasses and herb. They have been brought to the laboratory into plastic jars and preserved in Glycerin (Glycerol). Present study is based on occurrence of Aphid species in different crops of Thatta district and its surrounding area because Thatta possess variable habitat, geography and suitable environmental conditions, which are favorable for many crops and insect pests. Major cultivated crops are sugarcane, rice, vegetables, papaya and oilseed. Present study was conducted during October-January 2017 in which different fields were visited during seasonal crops. Total 3 species *Myzus persicae* (Sulzer), *Aphis fabae* and *Rhopalosiphum padi* belonging to genus *Myzus*, *Aphis* and *Rhopalosiphum* of Aphididae family were collected from brassica, Wheat and ornamental plants mostly in aphid colonies.

OCCURRENCE OF BRINJAL SHOOT AND FRUIT BORER (BSFB) IN BRINJAL FIELDS OF DISTRICT SANGHAR, SINDH

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Brinjal Shoot and Fruit Borer (BSFB) *leuciods orbonials* is the major pest of brinjal *Solanum melongena*. Brinjal is the one of the most important vegetable of south and South-East Asia. It is grown on over 678000 ha, with annual 10.50 million tons production. Present study was conducted in brinjal fields of Shahdadpur, District Sanghar Pakistan, from April to November 2017. Total one hector area was selected for study purpose. During present study we were found infestation of (BSFB) from September to November. Maximum infestation of fruiting bodies and shoots recorded during October and November. The prevalence of (BSFB) was first time observed in this area and growers are still confused to differentiate it from Pink boll worm.

DROUGHT STRESS AT POST ANTHESIS IN WHEAT UNDER CONTROLLED AND OPEN FIELD CONDITIONS SIGNIFICANTLY AFFECTS YIELD AND PROLINE CONTENT

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Drought is major constrain to wheat yield and all around causes noteworthy yield losses. Expanding wheat resilience to dry spell by screening diverse germplasm and incorporation of tolerant genes is a vital objective of the wheat breeding program. The present study was designed to screen drought tolerant genotypes among the diverse collection of CIMMYT bread wheat nurseries using yield traits and proline content. A total 98 CIMMYT wheat lines along with 10 local (Pakistani) high yielding varieties were screened under controlled and field conditions during cropping year 2014/15 and 15/16. For induction of drought stress, soil moisture content was maintained at 12.5% in both pot as well as field experiments. Phenological and yield traits were estimated along with proline content. Genotypes maintaining higher yield under stress and non-stressed environments had higher values of yield components. Results from correlation analysis showed that proline content had a weak correlation with grain yield and genotypes having higher yield values had high proline content. The present study reports 15 tolerant and five sensitive genotypes based on STI rank these lines can be incorporated into the breeding program.

EVALUATING THE EFFICACY OF DIFFERENT PESTICIDES AGAINST THE DUSKY COTTON BUG (*OXYCARENUS HYALINIPENNIS*) UNDER FIELD CONDITIONS

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Dusky cotton bug, *Oxycarenus hyalinipennis* Costa (Lygaeidae: Hemiptera) is a destructive pest of cotton. It sucks cell sap from leaves, flowers and from tissues, while lint quality is also affected by the dusky cotton bug (DCB) as it gets crushed during the ginning process. The pest is polyphagous in nature having multiple hosts. To manage insect pests in Pakistan, farmers mostly rely on the use of pesticide. So, the present study was designed to access the most effective pesticide against the studied insect under field condition. Three replications were carried out by using Randomized Complete Block Design (RCBD). Different insecticides were used such as Bifenthrin, Fipronil, Triazophos, Chlorpyrifos, Lambda-Cyhalothrin, Deltamethrin+ Triazophos and Clothianidin. Data was collected after 24, 48, 72 hrs and one week of spray. Statistically,

analysed data shows that most effective insecticide was Chlorpyrifos followed by Triazophos and Lymdda cyloahethrin, while among all the pesticide used Fipronil was the least effective against the DCB up-to seven days. So these insecticides can be recommended to the growers to control the said insect pest.

FOOD AND FEEDING HABITS OF INDIAN CRESTED PORCUPINE (*HYSTRIX INDICA*) IN DISTRICT BAGH AZAD JAMMU AND KASHMIR

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The Indian Crested Porcupine (*Hystrix indica*) is an important rodent pest species in the agricultural system of Pakistan. It causes huge economic loss as it feeds upon agricultural crops and other plants. Unfortunately, no formal data is available regarding this in AJ&K. This study examined the food habits of Indian Crested Porcupine (*Hystrix indica*) in District Bagh Azad Jammu and Kashmir. Total of eighty fecal pellets samples and stomach contents of twenty animals were analyzed. Total of thirteen plant species in winter, fifteen in spring, twenty in summer and fourteen plant species in fall were recovered from stomach contents. While fecal analysis revealed that thirteen plant species in winter, sixteen in spring, twenty in summer and eighteen plant species in fall were consumed by species. The stomach analysis revealed that seeds were preferred in three seasons with a percentage value of spring (35.4%), summer (28.2%) and in winter (24.8%). While the fecal analysis showed that stem was preferred in spring (57.2%), winter (41.3%) and fall (35.2%) and seed (36.7%) in summer. Among plant species *Zea mays*, *Diopyrus lotus*, *Melia azedarach*, *Lagenaria siceraria*, *Cucumis melo*, *Rumex obtusifolius*, and *Pinus wallichiana* were highly preferred. Berger-Parker diversity index indicated that the diet of porcupine was the most diversified (10.92) in summer season followed by spring (7.53), winter (6.53) and fall (2.95).

IMPACT OF HABITAT MANIPULATION ON RODENT POPULATION IN WHEAT CROPPING SYSTEM OF RAWALPINDI

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The rodents are widely distributed and are serious agricultural pest in Pakistan. During non-crop season wild vegetation provides shelter/cover and food to the rodent populations. Therefore, the present study was designed to investigate the impact of habitat manipulation by eliminating wild vegetation on field boundaries on rodent population. Six sites were selected in district Rawalpindi and randomly divided into two groups; wild vegetation was removed on the boundaries of fields of treated group while untreated group was kept as control. Burrow and rodent density was measured during sowing, tillering and maturity stages of wheat crop in treated and untreated sites. The results of burrows density revealed that higher ($P < 0.05$) number of burrows were present at untreated sites as compared to treated sites. The present study revealed occurrence of four rodent species in the following order of dominance: *Bandicota bengalensis* > *Nesokia indica* > *Tatera indica* > *Golunda ellioti*. It is concluded that habitat manipulation by eliminating wild vegetations on field boundaries during non-crop season can reduce the rodent's population. Moreover, habitat manipulation is a better non-chemical method of rodent control instead of expensive rodenticides to save farmer money that also protects the non-target species from poisoning.

MANAGEMENT OF THE TEPHRITID FRUIT FLY COMPLEX WITH INTEGRATED APPROACH**Hafiza Tahira Gul, Shafqat Saeed and Unsar Naeem Ullah***Department of Entomology, Muhammad Nawaz Shareef University of Agriculture Multan.*

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Tephritid fruit fly complex with their broad host range and massive population bursts results in damaging a variety of horticultural crops, and ultimately affecting the marketable produce. The pest has been active throughout the year, resulting in the severe damage to the central fruit crops of Pakistan. The management of this insect pest is widely dependent on the pheromone lures and some insecticide treatments. However, the studies have proved that an integrated approach for the management comes up with limiting the growth of insect pest population. For managing tephritid complex, a combination of cultural control (eradication plans for the infested fruits), biological control methods (use of entomopathogenic fungi and nematodes), chemical applications (new chemistry insecticides), baits (Protein Lures) and sterile insect technique (SIT) may prove to be useful. These area-wide approaches are providing a baseline towards bringing down the pest populations below the economic injury levels in the global scenario.

STUDY ON DIVERSITY, RELATIVE ABUNDANCE AND DISTRIBUTION OF GRASSHOPPERS IN ZEA MAYS CROP IN DISTRICT SIALKOT, PUNJAB, PAKISTAN**Suneela Nasrullah, Sajida Mushtaq, Sadia Maalik, Anam Sarwar and Mahwish Qamer***GC Women University Sialkot*

Agriculture is an extremely important sector of Pakistan's economy. It plays a vital role for economic development and growth in this country. Biodiversity plays a crucial role to maintain balance between pests and their natural animals providing better opportunity to control pest outbreak. A preliminary study was conducted to observe the diversity, distribution and relative abundance of grasshoppers in district Sialkot, Pakistan. *Zea mays* crop was selected due to its core importance as it is the third largest grown crop in the world. To fulfill our aim different areas were randomly selected for sample collection. Sampling was done fortnight for the period of six months. Grasshoppers were collected from each selected area during dawn and dusk, using sweep net, and handpicking method. The collected samples were preserved in wooden jars, and then identified. 20 species were present, belonging to 4 families and 14 Genera were observed. The members of sub-family Acrididae were most abundant 392 followed by another sub-family Oxyninae representing 341 members. The high value of Shannon Index showed greater richness and the evenness of species in the study areas. The value of Simpson Index (0.948) showed dominance in terms of more species diversity. *Hieroglyphus banian*, *Oxya hyla*, *Oxya japonica* and *Acrida exaltata*, with 189, 172, 169 and 128 respectively were the abundantly species whereas *Melanoplus bivittus* (39) was the least common species. In the month of April-July (71.40 %) maximum population were recorded. More population of grasshopper in the study area showed the need of time is proper pest management plans to minimize the economic losses and conserve biodiversity in maize.

ESTIMATION OF EXTENT OF DAMAGE AND LIFE CYCLE OF A PEST *PIERIS BRASSICAE* (LEPIDOPTERA: PIERIDAE) ON FOUR CROP PLANTS**Sadia Maalik¹, Shahnaz Akhtar Rana², Naureen Rana², Sajida Mushtaq¹, Naheed Bano¹ and Nazia Ehsan²**¹*Department of Zoology, GC Women University, Sialkot, Pakistan*²*Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad*

Photosynthesis is directly related with net primary production. The herbivores decrease photosynthesis and cause decrease in production. Quantitative analysis of consumption and utilization of host plants by insect herbivores is a commonly used tool in studying plant- insect interaction. The cabbage butterfly *Pieris brassicae* (Lepidoptera: pieridae) is one of the most destructive insect pests. So, it was taken to know its response on four

crop plants namely, bassica, cabbage, cauliflower and turnip. Larvae were provided with pre-weighed crop as feed to estimate the life cycle and extent of foraging. The length and weight of larvae along with weight of feed was calculated. Longevity was estimated by providing 20% honey solution. Each trial was replicated three times. One way analysis of variance with tucky contrast at 0.05 probability was applied to check the host preference. *P. brassicae* was found to be a destructive pest species of brassica by consuming 21.36 ± 0.72 g during its entire larval duration. This species showed least consumption on turnip (8.22 ± 0.60 g) with maximum larval duration of 17.67 ± 0.47 days. Pupal weight and percent adult emergence was directly correlated with food consumption. Such types of work is very helpful in planning Integrated Pest Management Programs (IPM).

EVALUATION OF *VALERIANA OFFICINALIS* AND *AZADIRACHTA INDICA* OILS AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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Plant oils are degradable, non-toxic and ecologically suitable for insect pest control. In this regard a study was carried out to evaluate comparative toxicological effects of neem plant (which is already documented for its toxicity against insects) with another indigenous medicinal plant oil, *Valeriana officinalis* (valerian) for their efficacy against red flour beetle, *Tribolium castaneum*. For the purpose, plant oils were tested to estimate their toxicity and efficacy as insect growth regulator (IGR). The plant oils were tested in three (0.100%, 0.050% and 0.025%) doses in comparison with control. Fifty grams of treated and untreated (control) wheat flour was kept in glass jars covered with muslin cloth. All trials were replicated five times, under the same temperature and humidity. No significant mortality was observed by valerian oil whereas neem oil caused maximum 68.75% mortality at 0.100% concentration after 72 hours. The plant oils reduced growth of the insect during metamorphic stages. By valerian oil maximum 88.35% larval inhibition and maximum 84.52% adult inhibition was observed at 0.100% concentration; whereas, by neem oil 68.42% larval inhibition and 98.55% maximum adult inhibition was observed at highest concentration, 0.100%. In view of determination of plant based management of grain pests, the findings of the study may be an addition for Integrated Pest Management of stored grain pests.

IMPACT OF SUBLETHAL DOSES OF INSECTICIDES ON INTRAGUILD PREDATION RESPONSES IN TWO COCCINELLID PREDATORS

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Intraguild predation (IGP) can occur among aphidophagous predators thus reducing their effectiveness in controlling crop pests. Among ladybirds, *Coccinella septempunctata* L. and *C. transversalis* are the most effective predators upon aphid, which is an economically important pest of canola crop. Understanding their likelihood to engage in reciprocal predation is a key point for conservation of biological control. Here, we aim to investigate, under laboratory conditions, the level of IGP between the two above mentioned aphidophagous species. In this study small canola leaves were treated with two sublethal concentrations (LC₁₀, LC₃₀) of two insecticides (Cypermethrin and Chlorpyrifos) and placed in a petri dish. The occurrence of IGP events was recorded after four hours. Predators were starved for 24 hours before test. Predation efficacy was evaluated for the following treatments/combinations: I- *C. septempunctata* adult, II- *C. septempunctata* larva, III- *C. transversalis* adult, IV- *C. transversalis* larva, V -*C. septempunctata* larva and *C. transversalis* larva, VI - *C. septempunctata* adult and *C. transversalis* adult, VII - *C. septempunctata* larva and *C. transversalis* adult, VIII- *C. septempunctata* adult and *C. transversalis* larva. There were five replicates for each treatments along with control treatment in which only of aphids were to evaluate the potential of escape and their natural mortality.

Predation efficiency of *C. septempunctata* and *C. transversalis* larvae and adult life stage alone and in combination was significantly affected by sublethal doses of chlorpyrifos and cypermethrin. In all combinations *C. septempunctata* was more superior to *C. transversalis* in showing intraguild predation. Larvae and adult *C. septempunctata* displayed higher predation efficacy as compared to *C. transversalis*. LC₃₀ of both insecticides exhibited considerably less predation efficacy as compared to LC₁₀ and control. Overall, the combination of larvae and adult life stages of *C. septempunctata* and *C. transversalis* showed significantly higher predation efficacy as compared to life stages alone.

**SCREENING OF SUSCEPTIBLE AND RESISTANCE CHICKPEA (*CICER ARITENUM* L.)
VARIETIES AGAINST POD BORER (*HELICOVERPA ARMIGERA* H.) IN BAHAWALPUR,
PAKISTAN**

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Chickpea (*Cicer aritenum* L.) is major consuming food in Pakistan. It can be grown easily as its production is very inexpensive and can be done even in unfavorable environmental conditions. The only threat to chickpea is the attack of pod borer (*Helicoverpa armigera* H.), which causes significant losses to this crop. Due to the developing trend of insecticide resistance in pod borer, it is becoming difficult to control it. Ten chickpea varieties (BRC-61, BRC 388, BRC-390, BRC-402, BRC-403, BRC-404, BRC-405, BRC-427, CHAKWAL-11 and PB 2008) were sown at experimental area of Regional Agricultural Research Institute in Randomized Complete Block Design (RCBD) with three replications. Screening of these varieties was done against the infestation of pod borer under non-sprayed conditions. It was recorded that BRC-405 and BRC-402 showed highly resistant response against pod borer with least damage percentage (7.577% & 9.352%) respectively as compared to all other tested varieties. CHAKWAL-11 showed high level of pod borer damage percentage (21.242%) than all other varieties.

**COMPARATIVE STUDY OF INSECTICIDAL ACTIVITIES OF BIO-PESTICIDE AND CHEMICAL
PESTICIDE ON THE GROWTH OF *MOMORDICA CHARANTIA* L. (BITTER GOURD)**

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The aim of this research is to correlate the insecticidal activities of bio-extract and synthetic extract on *Momordica charantia* L. (bitter gourd) plant. By treating the seeds of bitter gourd with bio-extracts and synthetic extract, the insect infestation was observed. Seeds treated with bio-extract insecticide produced a healthy growth of bitter gourd with ecofriendly nature and increases the fertility of soil, whereas the growth produced by the seeds treated with synthetic pesticides damaged them. The study was done in the Zoology lab of Jinnah University for Women, Nazimabad, Karachi from July to November 2017. The major and minor differences were found in the growth of leaves, stems and the duration of germination period. Sunlight and the area of experiment were also affected on the growth of plants. Bitter gourd is infected by several types of insect pests (Coquillett, 1849). Pakistan is one of those countries who are practicing synthetic pesticides in huge amount to enhance their production and in farmers there is lack of knowledge about the hazardous effects of synthetic pesticides (Munawar and Hameed., 2013). The bio-extracts use as a repellent, inhibit feeding of insects and helps to control the termites, beetles, aphids, flies, cut worms and other species of insects (Rahaman *et al.*, 2008).

ANTIFEEDANT AND TOXIC EFFECTS OF RED SEAWEEDS AGAINST PESTS

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Insufficient quantity of food, including the limited qualitative food, is the current challenge that the world is facing. The major factors affecting the food production are the water shortage, increasing human population, pollution and crop damage caused by insect pests mainly by feeding or by transmitting diseases. About 45% of the total food crop is lost by pests. The use of pesticides over decades to control pests limits its effectiveness, resulting in the increasing pest resistance and adverse environmental effects. Whereas, the plant-derived compounds are safer for the environment and efficient to control crop pests. Marine macro-algae is rich sources of natural chemicals widely spread in the World Ocean. Pakistan coast is also enriched with diversified marine macro-algae. In the present study, toxic and deterrent effects of red seaweeds were evaluated. Seaweeds were collected from Karachi coasts. Samples were dried, powdered and extracts were prepared using Soxhlet extraction of varying polarity. For pest infestation, plants were sown in the greenhouse for several weeks and major pests were separately cultured inside the cage. For bioassay, fresh leaves were cleaned, dried and 5cm discs were made and dipped for 5sec in five different concentrations. Leaf disc was placed on 1% agar bed. Insects were released and covered with a ventilated lid. Toxicity and antifeedant effects were assessed with 24-h interval. In the experiment, red seaweeds have proven significant antifeedant and toxic effects, showed the presence of insecticidal compounds that can be further isolated and identified.

EVALUATION OF DIFFERENT POTATO VARIETIES AGAINST POTATO APHID, *MYZUS PERSICAE* (SULZER) IN SWAT

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A research study was conducted to investigate the population abundance of *Myzus persicae* under field condition and to use antixenosis and antibiosis test in different potato varieties against *M. persicae* under controlled environment at Agricultural Research Institute (North), (ARI) Mingora, Swat, during spring, 2017. Bartina, Paramount, karuda, Roko, Delia red and Ronaldo potato varieties were arranged in Randomized Complete Block Design in the field with three replications, while in glass house these varieties were replicated ten times in Completely Randomized Design for antibiosis and antixenosis tests. Data regarding the overall mean number of aphids on different varieties showed significant difference among each other. The highest aphids infestation (2.7 Aphids leaf⁻¹) were observed on Delia red, followed by Karoda, Bartina, Paramount and Roko, where 2.5, 2.0, 2.0 and 1.8 Aphids leaf⁻¹, respectively. Lowest mean number of 1.6 Aphids leaf⁻¹ infestation was observed on variety Ronaldo. Initially the mean aphid population was low (0.7 Aphids leaf⁻¹), and infestation enhanced as time proceeded forward and highest infestation (3.3 Aphids leaf⁻¹) was observed at 4th week (18th April). After that, decreasing trend in aphids was absorb and the lowest number of aphid (1.6 Aphids leaf⁻¹) was recorded on last week 7th (10th May). Furthermore, the maximum yield (31.93 Tonnes (t) /ha) was found on variety Ronaldo followed by Roko (30.50 t ha⁻¹), Bartina (29.73 t ha⁻¹), Karoda (27.45 t ha⁻¹) and Paramount (27.31 t ha⁻¹). Minimum yield (26.61 t ha⁻¹) was found on variety Delia red. Negatively significant correlation were found between aphids population and total yield production. Overall variety Ronaldo showed excellent response toward aphids infestation and total yield production in t ha⁻¹. In antixenosis test, *M. persicae* showed no significant preference towards any tested variety, after 12, 24 and 48 hours. In antibiosis test variety Ronaldo showed significant resistant towards *M. persicae* compared to all the tested potato varieties.

**BIOEFFICACY OF ETHYL ACETATE EXTRACTS FROM PLANT MATERIALS ON MAIZE
WEEVIL (*SITOPHILUS ZEAMAI*S) (CURCULIONIDAE: COLEOPTERA)**

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Insect pests cause extensive quantitative and qualitative losses to stored grains and their value added products. Among the stored grain pests, *Sitophilus zeamais* (L.) is considered as one of the most destructive pests of stored cereals around the globe. Control of maize weevil is mainly achieved by the applications of fumigants. However, the excessive use of fumigants has led to the development of resistant populations, enhancing the need to develop alternative control measures. Plant derivatives are emerging as alternatives to traditional chemical insecticides as they carry rich sources of bioactive molecules. In the present study the ethyl acetate extracts of six different plants viz. Neem seed (*Azadirachta indica*), Bitter cress succulent fruit (*Caralluma tuberculata*), Garlic rhizomes (*Allium sativum*), Turmeric rhizome (*Curcuma longa*), Tumha fruit (*Citrullus colocynthis*) and Ak leaves (*Calotropis procera*) at six concentrations (5000, 10000, 15000, 20000, 25000 and 30000ppm) were evaluated against maize weevil for their effects on days to F₁ emergence, inhibition of F₁ adult emergence, percent infestation and grain weight loss, adult longevity and sex ratio. The newly emerged ten pairs of adult weevils were exposed to the plant extracts by grain treatment test in 200ml transparent plastic jars at constant conditions of 27±2°C, 65± 5% R.H in the laboratory of Entomology Department, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan. The results revealed that all the plant extracts carry biological effects against maize weevil compared to control. The significantly longest developmental duration, minimum number of F₁ adult emergence, percent infestation and weight losses were observed in grains pre-treated with the highest concentration of *A. indica* extracts followed by *C. longa* extracts while the shortest developmental duration, highest incidence and maximum grain damage was observed in untreated maize grains. Moreover, all the tested extracts significantly reduced the adult longevity of maize weevil compared to control. Moreover, the shortest adult longevity of maize weevil was recorded in maize grains treated with 30000ppm concentration of *A. indica* extracts whereas the longest adult longevity was recorded on untreated maize grains. The overall results of this study suggest that *A. indica* and *C. longa* extracts has potential insecticidal effects which might be used in pest control.

**EFFECT OF TRAP CROPS ON INSECT PEST INFESTATION AND PREDATOR
POPULATION ON COTTON CROP**

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Effect of trap crops on insect pest infestation and predator population on cotton showed that cotton plant alone suffered maximum attack of pests in comparison to cotton planted with different trap crops. The overall maximum population was recorded in cotton (sown as sole crop suffered highest infestation of jassid (2.61/leaf) followed by (1.67), (1.33) and (0.96/leaf) in cotton with sunflower, Bajra and maize. Similarly the maximum thrips population (11.95/leaf) was observed in cotton alone followed by (10.15), (8.53) and (7.44/leaf) in cotton intercropped with sunflower, Bajra and maize. The highest numbers of whitefly ((0.89 /leaf) was recorded with cotton alone followed by (0.52) and (0.42/leaf) in cotton with sunflower, Bajra and maize. Comparatively higher mean population of predatory bugs were recorded in cotton with maize (0.66, 2.36 and 0.93/plant), followed by *G. punctipes*, Orius and mystry bug. Maize was preferred hosts of whiteflies and jassid and attracted and reduced the pest stress on main cotton crop; whereas, sunflower and Bajra provided shelter to natural enemies which kept the pests of cotton below than cotton alone. The population of natural enemies varies with the temperature and relative humidity; however, in conditions.

EVALUATION OF OXIDATIVE STRESS IN *PISUM SATIVUM* IN RESPONSE TO DIFFERENT INSECTICIDES AGAINST *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE)

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Pea crop is attacked by many insect pests; however, pod borer, *Helicoverpa armigera* is the secondary but important pest of this crop. The most common approach to control this pest is the use of insecticides which is more effective and rapid method to control this pest. But excessive and uneven use of insecticides cause extreme stress on plants. Current study was planned to evaluate the efficacy of three different insecticides (bifenthrin, emamectin benzoate and lambda-cyhalothrin) at recommended dose against *H. armigera* and different stress related parameters in peas were also examined by studying antioxidant enzyme. Two local varieties (Aleena Gold and Climax) were sown at experimental area, Department of Entomology, University of Agriculture, Faisalabad by using factorial under Randomized Complete Block Design (RCBD). The samples from plants were collected from the field before and after application of insecticides on weekly basis. The lipid peroxidation rate, proline content and activities of antioxidant enzymes (CAT, SOD and POD) were examined. Results showed that all three insecticides effected the antioxidant enzymes activity, lipid peroxidation rate and proline contents in plants. Results of laboratory bioassay showed that emamectin benzoate proved itself more effective in term of management of *H. armigera* and gave 78% mortality while mortality was 76 and 71% by Lambda-cyhalothrin and bifenthrin respectively. As compared to other two insecticides, bifenthrin was more toxic for plants and more affected the antioxidant enzymes activity. The stress was observed in all time intervals but it was maximum in 2nd week after application. Emamectin showed less stress as compared to bifenthrin while lambda- cyhalothrin showed the least stress in comparison with emamectin and bifenthrin. To overcome this oxidative stress, jasmonic acid (JA) was used to reduce the stress which proved to enhance the antioxidant enzyme activity and non-enzymatic contents, which are lipid peroxidation rate and proline contents.

BIOTOXICITY OF LOCAL ISOLATES OF *BACILLUS THURINGIENSIS* FOR THE CONTROL OF MOSQUITO

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Bacillus thuringiensis is a naturally occurring facultative anaerobic, gram-positive bacterium forms spores containing crystals comprising one or more Cry or Cyt proteins that produces endotoxins that are selectively toxic to many mosquito and black flies. The present study was aimed to control resistant insects by isolating soil samples from different localities intended for effective mosquitocidal *cry2*, *cry4*, *cry9*, *cry11* positive *Bacillus thuringiensis* (*B.t.*). Control of various mosquito species at various developmental larval stages is a useful measure to mitigate transmission of pathogens by mosquito vectors. Various species of *B.thuringiensis* reported to be toxic against mosquitoes, black flies. Hundred Strains have been isolated from different localities of Punjab from diverse habitats, including soil, insects, stored-product dust, and deciduous and coniferous leaves. 15 *B.t.* strains were screened, 75% were from dry, leaf litter, garden soil samples, 16% from animal waste and 10 % from moist soil of crop area. Genomic DNA was isolated and a DNA fragment of 650bp, 439bp of *cry11*, *cry4* gene was amplified by PCR respectively. Seven *Bacillus thuringiensis* were brought to be positive for *cry11* and *cry4* gene. The 16S rDNA study exposed that these screened *B.t* confirmed 99% homology with *B.t.* serovar *tolworthi*, *B.t.* str. *Al Hakam*, *B.t.* serovar *thuringiensis*, *B.t.* serovar *konkukian*, and *B.t.* serovar *Chinensis*. *B.t.* serovar *Indiana*, *B.t.* serovar *kurstuki*. The toxicity bioassays with *B.t.* spores proved that six *B.t.* isolates harboring *cry11* and *cry4* genes (*viz.*, NF1*B.t.*, 2,3,4,5,6,7) were most toxic to 3rd instar larvae of mosquito, *Aedes aegypti*, *Anopheles stephensi*. Among six *B.t.* isolates, GCU-DAB-NF5 was found the most toxic and was isolated from moist soil containing a Dirty sewage water LC50 is 522.027±0.17 µg/ml against *Aedes aegypti* (third instar larvae) and showed 100% mortality at 1000µg of spores/ml. The

positive control HD-500 showed 94% mortality. It was found that LC₅₀ (522 µg/ml) of GCU-DAB-NF5 is quite less than HD500 LC₅₀ (673 µg/ml). So, GCU-DAB-NF5 is more toxic as compare to HD500. Among these *Bt* 2 shows the same LC₅₀ higher than HD500 against *Anopheles stephensi* harbouring *cryII* and *cry4* gene. All isolates did not show the same level of toxicity which reflects the variation in expression level of *cryII*, *cry4* gene present in local *B.t.* isolates. The study provides a convenient method which is time saving and economical. This study recommends that *B. thuringiensis* at spore stage provides good mortality percentage.

**BIO-EFFICACY OF SOME BOTANICALS AGAINST RUST RED FLOUR BEETLE,
TRIBOLIUM CASTANEUM (COLEOPTERA: TENEBRIONIDAE)**

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Tribolium castaneum is sever insect pests of stored grain products. The current study was targeted to evaluate toxic and growth inhibitory potential of plant derived insecticides (extracts) (*Azadirachta indica*, *Murraya exotica*, *Eucalyptus globulus*, *Trachyspermum ammi* and *Teminalia chebula*) on 5, 10 and 15 % concentrations against the *T. castaneum*. The findings of mortality bioassays revealed that maximum mortality 16.11% in case of *A. indica* at 15% concentration while minimum (2.78%) was recorded in case of *T. chebula* extract wit 5% concentration. Growth inhibition results showed that highest pupae inhibition was noticed in case of *E. comeldulensis* (70.21%) while *T. ammi* and *T. chebula* were both equally effective with (54.82%). In case of adult inhibition bioassay, (66.67%) inhibition was observed in case of *E. comeldulensis* greater than *A. indica* (58.82%) and *M. exotica* (54.90%) whilst least inhibition was recorded in extract of *T. ammi* (52.08%) and *T. chebula* (50.00%). Therefore, the use of botanicals is eco-friendly tactic and can be used in an integrated manner with other control measures for the efficient management of insect pests of stored commodities.

**TRANSOVARIAL AND OVICIDAL EFFECT OF LUFENURON ON *TRIBOLIUM*
CASTANEUM (HERBST) (COLEOPTERA: TENEBRIONIDAE)**

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The Chitin synthesis inhibitor, lufenuron was assessed for ovicidal and reproduction inhibitory effects at different concentrations for two strains of *Tribolium castaneum* in separate laboratory bioassays. In the first experiment, all the tested concentrations of lufenuron have no effect on adults that were exposed to lufenuron-treated flour, but at 0.08 ppm concentration of lufenuron, 56.9 and 61.7 percent reproductive control was obtained in the Multan (ML) and Faisalabad (FD) strains, respectively compared to control. Egg sterility of 85.4 and 89.3% taken place at 0.08 ppm in the ML and FD strains, respectively compared to control. Subsequent development of F₁ larvae, pupae and adults from hatched eggs were also severely affected. In a separate experiment, 41.2 and 43.6% egg sterility taken place in ML and FD strains, respectively when eggs laid by unexposed beetles were directly exposed to 0.08 ppm concentration of lufenuron. Further, subsequent development of larvae, pupae and adult emergence was also inhibited. However, the inhibition effects due to direct egg treatment were not as significant as those obtained due to the adult treatment. Results show that lufenuron can be a potential product for pest management in mills, warehouses and food storage facilities.

TO CATEGORIZE THE ANTIBIOSIS AND TOLERANCE IN EIGHT DIFFERENT CULTIVARS OF POTATO AGAINST *M. PERSICAE* (HOMOPTERA: APHIDIDAE)

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Crop resistance is defined as that it is the genetic character of plants to avoid the attack of insect pest to reduce the plants damages. In the Integrated pest Management plant resistance is the new and most important model recognized by the national and intragovernmental bodies for selection of best resistant varieties and pest management. The category of resistance antibiosis influence the growth and development of insect pest due the presence of some chemical and physical features in the plant. Tolerance is the category of resistance of a plant to show the ability to withstand to herbivore without any decline in yield. In antibiosis the single adult aphids (P1) were caged on potato plant leaf and were observed twice a day up to the production of first nymph (F1). The mother aphid were moved to next leaf on the same plant until the nymph produce its first progeny the time and date are recorded. In tolerance pairs of plants were made in completely randomized design, one plant infested with known numbers of aphids and pair plant leave uninfested and caged both the plants with nylon mesh cages. The plants removed after 15 days or the susceptible plants show leaf rolling curling etc. The plants cut at soil surface pouch in aluminum foil and dry up to 72 hours in oven then weight. The cultivar Rocco delay the first nymph production of aphid and take 4.86 days while Desiree 2.69 days. Tolerance show by the cultivar Desiree having the weight 0.50 g. Chlorophyll content check by SPAD meter the cultivar Desiree have more content 0.67. This new heritable resistance in a potato cultivars may serve for further breeding and research purpose.

FOOD HABITS, FEEDING SEASONALITY AND ASSESSMENT OF DAMAGE INFLICTED BY THE SMALL KASHMIR FLYING SQUIRREL (*HYLOPETES FIMBRIATUS*) IN DISTRICT BAGH, AZAD JAMMU AND KASHMIR

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The Small Kashmir Flying Squirrel (*Hylopetes fimbriatus*) is a frugivore rodent, hence causes substantial economic loss. The current study highlights the harmful effects of flying squirrel on fruit trees in district Bagh, Azad Kashmir during a period stretching from November 2016 to November 2017. The study area was divided into ten study sites and forty-thousand-meter square area was visited. The damage was assessed by counting numbers of affected trees (wild and planted). The stomach content and fecal pellets were also analyzed to find the food habits of the species. The results of direct visualization revealed that the squirrel species causes 51.90% fruit loss from fifteen different fruits species. In Summer, Winter and Fall cultivated fruits while during Spring season wild plants were deteriorated. During Summer (44.85%), Winter (31.38%), Fall (37.53%) and Spring (83.21%) fruits were ruined by the species. From collected four stomachs samples of Fall Season, the parts of eight (n=08) plants species were obtained. Highest Relative Frequency was found of *Pyrus communis* (49.32 %). Only three plants species, some unidentified material and Leaves were recovered from stomach content during Spring. *Quercus incana* (30.28%) have maximum Relative Frequency. In Summer season, the highest Relative Frequency was found for *Juglans regia* (44.33%). Similarly, in Winter Uppermost Relative Frequency was recorded for *Pyrus communis* (49.32%). The food habits of species were also analyzed through fecal pellet analysis. A total of four hundred fecal pellets (ten pellets from each study sites so hundred in each season) were analyzed in different seasons. The fragments of five fruits species were recovered from Winter scats. The highest fragments were found of *Prunus padis* (26%). From Spring scats only three Wild plants were obtained and the maximum particles were found of *Pinus wallichiana* (46.67%). In Summer scats, parts of six fruits were gathered. Most of the particles were belongs to *Juglans regia* (32.94%) and lowest belongs to *Prunus domestica* (2.35%). Whereas the scats of Fall contain fragments of eight different fruits species. Highest

parts were of *Juglans regia* (22.13%). The morphometrical analysis (length, width and weight) of scats were also carried out to identify the seasonal effects of food items. The largest scats size was found in Summer while smallest size was recorded in Fall season. Similarly, the widest fecal pellets were also measured in Summer and least width were found in Fall season. The highest weight of scats was found in Summer and lowest were found in Spring season. The area needs further exploration.

TO STUDY THE TOXICITY OF BIO-PESTICIDE AND CHEMICAL PESTICIDE ON *TRIBOLIUM CASTANEUM* (HERBST, 1797)

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In the present work the toxic effects of bio-pesticides and synthetic pesticides were observed against *Tribolium castaneum* (Herbst, 1797) which is a pest of stored maize and a variety of stored products. Regression models for toxicity of insecticide and test species were also developed to calculate the lethal concentration after 24 hours treatment on doses of 1ml, 2ml, 3ml, 4ml and 5ml by using different concentrations of bio-pesticides and synthetic pesticides. This experiment was carried out to determine the comparative toxicity of both the insecticides. Experiments were conducted on adult beetles i.e. *T. castaneum* to find out their LC₅₀. For the treatment of adult beetle five different doses of each pesticide was prepared from their stock solutions. Insects infest grains after harvesting, cause economic losses to production, grains and food industry. The synthetic pesticides used to control agricultural stored-product insect. The grain industries want to diminish the use of synthetic pesticides because of insecticide deregulation, resistant populations and consumer concerns over insecticide residues (Korunić & Rozman., 2010). *T. castaneum* considered one of the major stored-product insects which cause significant losses of grains and its byproducts throughout the world and the most frequent control of these insect and other stored-product insects is usually by fumigation with methyl bromide (Batta & Safieh., 2015).

HOW DICHLOROMETHANE AND METHANOL NEEM SEED EXTRACTS AFFECT COTTON MEALYBUG (*PHENACOCCLUS SOLENOPSIS*) MORTALITY AND EMERGENCE OF AN ENCYRTID PARASITOID (*AENASIUS BAMBAWALEI*) UNDER LABORATORY CONDITION

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The toxic effects of Neem seed extracts in methanol and Dichloromethane were studied under laboratory condition against three different life stages of cotton mealybug *Phenacoccus solenopsis* Tinsley. The experiment was laid out under CRD with two main treatments, each with 3 different concentrations and a control, replicated five times. Results showed that among all treatments/concentrations the highest percent mean mortality of all three stages i.e. 1st, 2nd instars and adult was recorded in 3% concentration of methanol neem seed extract. In all these treatments significant mortality ($p > 0.05$) was recorded during the whole weeks as compared to control ($14 \pm 1.87\%$), however Dichloromethane extracts showed non-significant ($p < 0.05$) results among each other but were significantly different from control (15 ± 1.58). Similarly all these treatments were checked for compatibility with parasitoid *Aenasius bambawalei* Hayat, which showed non-significant effect of these treatments on percent emergence from the pupae. From this experiment it was concluded that the mortality is directly correlated with dose and time. All treatments showed control but 3% methanol neem seed extracts were significantly different from others treatments. Therefore the concentration (3%) is recommended. However, further study needs to be conducted on field level to confirm these experiments.

EVALUATION OF REPELLENCY OF *ANNONA SQUAMOSA* (L) LEAVES POWDER AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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Materials used for bait formation and taste additives to attract rodents may attract the insect pests, resulting in insect infestation and finally spoilage of the bait. Many researchers recommended for the addition of invertebrate antifeedants in anticoagulant baits. Some plants with antifeedant and insecticidal activities against insect pests are tested by some earlier researchers; however there is a dearth in the bio efficacy of many indigenous plants against the insect pests. Custard apple (*Annona squamosa* L.) extracts have revealed potential for pest control against a range of insect pests. In some previous studies extracts of custard apple were found very effective against crop pests; In view of these studies, a study on the toxic effects of custard apple (*Annona squamosa*) leaves powder against *Tribolium castaneum* was carried out. For the purpose anticoagulants bait was prepared by mixing wheat flour, broken rice, additive (egg) and poison (brodifacoum 2.5% master mix by Kukbo Pharma Co; Korea) in a ratio of 46:46:3:5 (Pervez *et al.*, 2000, 2003). For testing custard apple leaf powder, wheat flour, broken rice, additive (egg), poison and neem seed powder were mixed in a ratio accordingly. The powder was mixed in wheat flour in three doses, 2.00%, 1.00% and 0.50%. Petri dishes were partitioned in two equal halves using hardboard. One partition was filled with 20 gram plain bait, the other was filled with 20 gram treated bait. The partition was removed and 20 insects were released in the middle. The settled insects were counted after one hour and six hours for five consecutive days. The repellency was observed up to second, fourth and eighth weeks. Fresh insects were used in all tests, using the same bait (treated and untreated). Evaluation of weekly repellency was carried out by comparing the results. The leaf powder showed highest repellency, 74.45% in the first week that gradually decreased to 62.00% in the eighth week at 2.00% dose; 63.52% in the first week that gradually decreased to 51.32% in the eighth week at 1.00% dose and 39.82% in the first week that gradually decreased to 25.21% in the eighth week at 0.50% dose. The study may be useful in utilization of custard apple leaves powder for the protection of anticoagulant baits from the infestation of red flour beetle, *Tribolium castaneum*.

COMPARATIVE STUDY OF INSECTICIDAL ACTIVITIES OF BIO-FERTILIZER AND CHEMICAL FERTILIZER ON THE GROWTH OF *ALLIUM SATIVUM* L. (GARLIC)

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The aim of this study is to compare bio-fertilizer, chemical fertilizer with control in order to know how bio-fertilizer is useful for the growth of *Allium sativum* L. (garlic) crop and compared it with the chemical fertilizer and control. The study was conducted on East Karachi in dry and main rainy season (March 2017 to July 2017) to assess the effects of bio-fertilizer and chemical fertilizer on growth of garlic. The properties of chemical fertilizers and bio-fertilizers are different, and every kind of fertilizer has advantages as well as disadvantages according to crop growth and soil fertility. Garlic has higher nutritive value than other crops in addition to carrying antibiotics like garlicin. (Maly *et al.*, 1998). Today, efforts to obtain higher yields and good quality of garlic have led to the application of various types of fertilizers that have dissimilar concentrations of plant nutrients and therefore affect the growth and yield of the crop differently. For higher yield and best quality of garlic can be enhance by increase nitrogen and sulphur application strategies as influenced by the source of N and S, as well as rates and times (Luo *et al.*, 2000). Therefore, increasing garlic yield and improving bulb quality are the desired attributes for growers, traders as well as consumers. (Marschner, 1995). The treatments consisted of controlled sample, three replicas of bio-fertilizer (organic) and three replicas of chemical fertilizer (inorganic). The morphological characters and pest infestation of garlichave been observed at different growth

stages. Prepared 7 pots in which 3 for bio-fertilizer and 3 for chemical fertilizer and 1 for control. Weight the soil (baalumitti) for each pot; weight bio fertilizer for 3 pots, and chemical fertilizer for 3 pots and weight cow dung for controlled. Mix soil with bio fertilizer and fill three replicas. Now sow the garlic .The same method is used for the each replica of chemical fertilizer and also for control. Now water the plants. Place the pots in sunlight. Observe the growth of plants and infestation of pests in each pot weekly. This process will be continuing for 6 months.

SURVEILLANCE FOR TRIPARTITE COMPLEX OF APHIDS, BARLEY YELLOW DWARF VIRUS (BYDV) AND WHEAT UNDER DIVERSE AGRO-ECOLOGIES OF KHYBER PAKHTUNKHWA PROVINCE OF PAKISTAN

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Surveillance for aphid-BYDV-wheat complex was carried out in ten districts of southern, central and northern zones of Khyber Pakhtunkhwa where 1820 acres of wheat crop was inspected and field data was recorded along with sampling of aphids and suspected BYDV infected samples for further lab analyses. Four aphid vectors (*Rhopalosiphum padi*, *R. maidis*, *Schizaphus graminum* and *Sitobion avenae*) and a BYD Virus-PAV were identified/diagnosed. In northern zone districts, *S. avenae* was identified up to 96% while in southern zone districts, *S. graminum* was detected up to 65%. Similarly, *Rhopalosiphum padi* reached to 52% in the central zone, while, *R. maidis* up to 36% in southern zone districts. Impact of BYD disease was maximum in the southern zone which was followed by central zone and northern zone. Zonal suspected BYDV infected leaves samples were indexed using Double Antibody Sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Results revealed that 91% of the tested samples were indexed as BYD Virus-PAV positive in the central zones followed by 84% in northern zone and 67% in the southern zone. Keeping in view the situation, resistant sources for both aphid and BYDV-PAV are needed to be explored to guard wheat crop against aphid-BYDV-wheat complex. Furthermore, continued R&D efforts are needed to study complete profile of aphid vectors - BYDV/CYDV in wheat and other cereals.

SECTION – III

ENTOMOLOGY

NEW RECORD *PICROMEROUS ORIENTALIS* (HEMIPTERA: PENTATOMIDAE: ASOPINAE) FROM KHAIRPUR, SINDH, PAKISTAN

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Picromerous orientalis (Rishi 1973) (Hemiptera: Pentatomidae: Asopinae) is a most important predatory stink bug produce a unpleasant smell distributed in all oriental region, they are predator on different larva's Coleoptera, Hymenoptera and Lepidoptera, *Picromerous orientalis* has been first time reported from the various localities of Punjab, KPK Mardan of Pakistan (Nazeer Ahmed Rana 1985), identify on the basis of external morphology and internal anatomy dissection male, female genitalia (spermtheca, spermathecal bulb, aedeagus, paramers, pygophore) and draw the diagrams. *Picromerous orientalis* first time reported from the Khairpur district of Sindh Province Pakistan.

NEW RECORD OF CHEWING LICE (PHTHIRAPTERA: INSECTA) ON GUINEA FOWLS (AVES: GALLIFORMES) FROM SINDH, PAKISTAN

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The Chewing lice (Insecta: Phthiraptera) are the common ectoparasites hosted by different birds of family Phasianidae (Aves: Galliformes) and have high capability to develop host specificity with their host. They have severe effects on health of their hosts directly and as well as indirectly. They cause various diseases and play a role as vector of some bacteria and helminthic worms. Presently, one type of large sized galliforme bird, Guinea fowl *Nomida meleagris* was selected for collection, identification, population density and rate of infestation of chewing lice from different regions of Sindh, Pakistan. 15 Guinea fowl *Nomida meleagris* were collected and brought into the laboratory. The study was conducted from April 2015 –March 2016. Fowls were kept on white paper sheet for about 30 minutes and sprayed with Permethrin powder in their wings. The infested birds were tagged with identity rings to check their lice after interval of 2 to 3 weeks. The chewing lice species were collected and preserved in 70% ethanol. The permanent microscopic slides were prepared with Canada balsam as standard method of preservation. Currently 130 chewing lice were recovered belonging to 3 genera and 4 species. All Chewing lice species are large to small size, pale yellow in color strong and stout body. These species and their Prevalence are 19.23% for *Menopon gallinae* (Linnaeus, 1758), 23.84% for *Menacanthus stramineus* (Nitzsh, 1818), and 22. 30% for *Menacanthus abdominalis* (Piaget, 1880) of family Menoponidae and 34.61% for *Goniocotes gallinae* (de Geer, 1776) of family Philopteridae. All these four species were reported first time as new host record and new locality record from the study area.

MORPHOLOGICAL IDENTIFICATION, BIOLOGY AND OVIPOSITIONAL SUBSTRATES PREFERENCES OF BROWN LACEWING, *SYMPHEROBIUS BARBERI* (NEUROPTERA: HEMEROBIIDAE)

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The brown lacewing (BLW) *Symphorobius barberi* is reported as an important predator of several insect

pests from Asia, Europe and America but never tested against the Asian citrus psyllid (ACP), *Diaphorina citri*, vector of huanglongbing or citrus greening disease. Adults of *S. barberi* were equally effective in consuming *D. citri* eggs and nymphs under both light and dark conditions. *D. citri* and *E. kuehniella* diets were equally suitable for development and reproduction of *S. barberi* except for slightly prolonged larval development on a *D. citri* diet with little more fertility 65% on *D. citri* diets compared to *E. kuehniella* 52%. Adults *S. barberi* lived long on diets of *D. citri* eggs or *E. kuehniella* eggs and were significantly differed when provided these diets in combination with nectar honey. *S. barberi* female preferred to lay eggs 64% on dimpled paper compared to substrates of plane paper, leaves of citrus, Murraya, eggplant and cantaloupe. These findings indicate potential usefulness of *S. barberi* against *D. citri* and warrant field release and evaluation. Additionally it is also concluded that this voracious predator may be reared on commercial basis by using the tested diets and substrates.

BOMBUS HAEMORRHODALIS ABUNDANCE AND POLLINATORS DIVERSITY IN RAWALPINDI/ISLAMABAD AND NARAN KAGHAN VALLEY, PAKISTAN

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Indigenous bumblebee *Bombus haemorrhoidalis* is only *Bombus* pollinator of Lower Northern Pakistan and playing key role in wild pollination in this area. Present study was designed to explore the relative abundance of *B. haemorrhoidalis* in comparison with other common pollinators and their diversity. Monthly surveys were conducted from all Rawalpindi/Islamabad and Naran Kaghan Valley during the years 2012 and 2013. Hymenoptera insect order was found as most abundant pollinator group with six species followed by Lepidoptera with five species. *Syrphus* sp was recorded with maximum abundant after *B. haemorrhoidalis* from all study locations. Maximum pollinator's diversity indices were found in Narna and F9 Park areas. Such ecological information is important to get their possible utilization in further biological experimentations, commercial pollination services and environmental conservation. This study will also be helpful to conserve this only *Bombus* pollinator in future in this area.

EFFECT OF FRESH POLLEN PATTLES AND POLLEN PATTIES WITH DIFFERENT CONCENTRATION OF SUGAR AND HONEY SOLUTION ON LIFE HISTORY PARAMETERS OF BUMBLEBEE, *BOMBUS TERRESTRIS*

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Bumblebees with buzz pollination phenomena are most important and efficient pollinator than honeybees. These bees play major role for pollinating many crops especially grown in greenhouse and glasshouse e.g. tomato, pepper, cucumber ect. To maintain their colony development and colony structure these bees required pollen and nectars for dietary needs. Here we investigate whether fresh pollen pellets and pollen patties with 40%, 50% and 60% of sugar and honey solutions have any effect on life history parameters of *Bombus terrestris*. Finding showed that pollen patties with 40% sugar solution exhibit best results at colony initiation stage with early pre-oviposition (6.6 ± 0.97), maximum numbers of egg beads at first batch (1.4 ± 0.22) and earlier emergence of first workers (28.3 ± 1.02). At colony foundation stage, pollen patties made with 50% sugar solution were considered suitable for the colonies to reach earlier at colony foundation stage (51.4 ± 2.53), late start of switch point, and early new daughter queen emergence. Similarly, at colony maturation stage,

pollen patties made with 40% sugar solution was best than other pollen patties with different concentrations of sugar and honey solutions on all parameters including total number of emerged males, daughter queens workers, competition point and mother or foundation queen longevity. Overall results showed that at colony initiation stage and colony maturation stage pollen patties made of 40% sugar solution is best and at colony development stage pollen patties with 50% sugar solution is best from all others fresh pollen and patties with different concentration of sugar and honey solution for continuous rearing of bumblebee under controlled laboratory conditions.

FIRST RECORD AND REDESCRIPTION OF *CARPOCORIS PUDICUS* PODA (1761) (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE: CARPOCORINI) FROM HYDERABAD REGION.

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Carpocoris pudicus Poda(1761) are small sized stink bugs belonging to order Heteroptera (Carpocorini) body ochreous colour, with stripes on head, pronotum, scutellum and striped connexiva. The species is redescribed on the basis of morphological characters, especially colour, shape of head, pronotum, scutellum, antennal segments and internal male genitalia (pygophore, paramere and aedeagus) and female genitalia (terminalia and spermatheca). A general description of *C. pudicus* is also given. The species of Carpacorini (stink bugs) is new record of Hyderabad region.

ROLE OF COCKROACHES AS CARRIERS OF BACTERIAL PATHOGENS DETECTED FROM PUBLIC HOSPITALS IN QUETTA, BALOCHISTAN

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Cockroaches (Dictyoptera:Blatodea) are potential vectors of pathogenic organisms, *usually found in contaminated environment from where they acquire nutrient. The nasty habitat makes them ideal mechanical vector of infective microorganism. In hospitals they feed on human viscera, sputum, blood, starch-like products, garbage and sanitary wastes. In the present study 360 cockroach specimens from the three genera and species viz Periplanata americana, (American cockroach) Blatella germanica (German cockroach), Blatta orientalis (Oriental cockroach) were collected from hospitals of Quetta city include Civil hospital, Bolan Medical Complex and Benazir hospital. Of the total 360 specimens 85% were positive with bacterial load, while 15% were negative. The American cockroach, P. americana was the most abundant (43.3%) among the three cosmopolitan cockroach species than B. germanica (34.1%) and B. orientalis (22.5%). Among the three hospitals, civil hospital was found more contaminated (29.2%) than Bolan medical complex (28.3%) and Benazir hospital (27.5%). The results revealed that P. americana was highest bacteria carrier (54.2%) than B. orientalis (27.5%) and B. gremanica (18.3%). Male cockroaches were found more pathogenic carrier (60%) than female cockroaches (25%). The obtained dilution from external surface and gut of cockroach were streaked on selective and differential media incubated at 37 °C for 24 hours. Bacteria of medical importance (nosocomial) were isolated and identified by biochemical tests and Gram staining to evaluate the effect of antibiotics against them. These include Escherichia coli, Klebsiella spp., Salmonella spp., Shigella spp., Clostridium spp., Staphylococcus aureus, Citrobacter spp., Enterobacter spp., Pseudomonas spp. and Listeria spp. Among these bacterial pathogens from external surface; E. coli indicated highest percent (77.5) prevalence and Listeria spp. showed least percent (4.1) ratio than those from GIT (15%) and (0.8%). Among applied antibiotic discs tested in the present study, Ciprofloxacin and Colistine sulphate were highly effective against all bacteria detached both from the external surface and gut. Tetracyclin was also found to be impressive against all isolated microbe species while resistant against Staphylococcus aureus, Shigella spp. and Pseudomonas spp.*

From these results it is concluded that cockroaches are significant mechanical carrier in hospitals environment, transfer various epidemic diseases causing pathogens subsequently a serious threat to human life.

TAXONOMIC HISTORY OF FORMICIDAE (HYMENOPTERA) IN PAKISTAN

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Family Formicidae play an important role in agriculture and are known as a pest, predator and other relationships. The taxonomic study of ants is neglected and poorly investigated from Pakistan but it has rich ant fauna, most of the part has been studied before the creation of Pakistan during British Rule including; Bingham (1897), Forel (1904), Manózi (1939), Eidmann (1942); later an expedition undertaken by Brown (1955) in Baluchistan and Sindh Provinces. Haji (2008) worked on house hold ants of Karachi, Pakistan. More recently Umair *et al.* (2010) described the ants from Potohar plateau of Punjab Pakistan and Ahmed *et al.* (2013) provided description on ants of Quetta, Baluchistan. Further, several species of ants described by various authors is available in scattered publications. From Pakistan 130 species are known under 40 genera and six subfamilies; Dolichoderinae, Dorylinae, Formicinae, Myrmicinae, Pseudomyrmecinae and Ponerinae. Pakistan occupies an important geographic position in old world and represents elements of Ethiopian, Palearctic and oriental zoogeographical regions. Ethiopian runs along southern coastal areas of Sindh and eastern Makran in Baluchistan, its Palearctic is continuous with those of Iranian Baluchistan, eastern Afghanistan, north western and eastern China, the oriental region is continuous with those of Indian Punjab and Rajasthan (Qadri 1968). More research on family formicidae is needed which will help to understand the current status of ant fauna in Pakistan, and will provide a basic knowledge on further research of ants. 38th congress of zoology (International) February 27 – March 1, 2018 Department of Zoology, University of the Punjab Lahore

ECONOMIC HETEROPTERA IN LOWER SINDH

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Sindh is divided into three climatic regions upper, middle and lower. In lower Sindh mostly sugar cane, banana, sunflower, rice, wheat, papaya and other crops are grown, maximum temperature reaches about 35–38 °C, these crops are invaded by numerous heteroptera, some feed directly on crop, whereas, others invade crops in search of their prey and their by recognized as agents of biological control. Commonly member of heteroptera are called as bugs and can be recognized by their forewings, which are half membranous and half hard. Suborder Heteroptera comprises 40,000 members TOLWP, (2005), Shaeffer (2000) stated that 40% bugs are beneficial and 60% are harmful. During present investigations in lower Sindh several members of heteroptera were collected, which revealed economic families including; coreidae, miridae, geocoridae, pentatomidae, nepidae and gerridae. Bugs with sucking mouthparts attack on crops by sucking sap of plants and other help us by sucking the sap of their prey and causing death of insects examples include assassin bugs under family Reduviidae. Big-Eyed Bugs are also predatory under family geocoridae which consists of 14 described genera; most species are in *Geocoris* and *Germalus*, and these include all the known economic species. The genus *Geocoris*, which is worldwide in distribution, included 124 described species, most described species, of these 20% occur in the Oriental region. Yet in our locality we are only aware of one species of *Geocoris*. Most of the previous work was conducted on family Pentatomidae, there is a great need of work to be conducted on heteroptera as a whole in Sindh. The audience will have insight to the currently found heteroptera from lower Sindh, habitus images of recently collected species will be provided. 38th congress of zoology (International) February 27 – March 1, 2018 Department of Zoology, University of the Punjab Lahore.

**BASED ON PATHOGENIC POTENTIAL AND PREVALENCE OF TURKESTAN
COCKROACH (*BLATTA LATERALIS*) CAPTURED FROM HUMAN DWELLINGS
IN QUETTA CITY, BALUCHISTAN**

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The Turkestan cockroach, *Blatta lateralis* (Walker) has been identified as an important specie which is replacing other cockroach species including German (*Blatta germanica*) and Americans cockroaches (*Periplaneta americana*), in Quetta City. Total 112 houses were being visited and 147 Turkestan cockroaches had been collected out of which 72% were found positive for carrying infectious bacteria. A total of 6 pathogenic bacteria including one Gram negative and five Gram positive bacteria were isolated from these cockroaches. We performed biochemical tests in order to identify the isolated bacteria. The internal cockroach bacterial contaminations (72.28%) were recorded higher than entire surfaces. Higher percentage of bacterial pathogens were found significant ($p < 0.000002$) in female cockroach (56.87%) than male ones (43.12%) at probability value 0.05. Our results suggest that most isolated bacterial species showed sensitivity to ciprofloxacin (an antibiotic) compared to other antibiotics used alongside. *Pasteurella* spp. completely failed to show resistivity against all antibiotics, while *Shagella* spp. showed highest resistivity against all antibiotics. Most severe infectious conditions were developed on intravascular inoculation of isolated *Pasteurella* spp. into mice body.

**COLLECTION AND IDENTIFICATION OF ANTHOPHILOUS FLIES
(DIPTERA) OF TANDOJAM**

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For current research anthophilous flies specimens were collected from various types of flowers at Tandojam and their surrounding areas. Collected specimens were further identified at Postgraduate Insect Systematic Laboratory Sindh Agriculture University Tandojam. In present study total 57 members of the order Diptera Linnaeus, 1758 from flowers found in the vicinity of campus. During the course of identification of anthophilous flies 07 species were discovered under two families; Syrphidae Latreille, 1802 and Calliphoridae Brauer & Bergenstamm, 1889. Under family Syrphidae total 05 flies from various flowers under two tribes were collected; Eristalini Newman, 1834 with 3 species, *Eristalinus sepulchralis* (Linnaeus, 1758), *Eristalinus* (*Lathyrrophthalmus*) *aeneus* (Scopoli, 1763) and *Eristalinus* (*Lathyrrophthalmus*) *arvorum* (Fabricius, 1787); and under tribe Syrphini Latreille, 1802 02 species, *Episyrphus balteatus* (De Geer, 1776) and *Sphaerophoria scripta* (Linnaeus, 1758). Family Calliphoridae with 02 species; *Pollenia rudis* Fabricius 1794 under subfamily and tribe Polleniinae and Polleniini; *Lucilia caesar* (Linnaeus, 1758) under subfamily and tribe Luciliinae and Luciliini. 38th congress of zoology (International) February 27 – March 1, 2018 Department of Zoology, University of the Punjab Lahore

**COLLECTION AND IDENTIFICATION OF BARK BEETLES (COLEOPTERA
CURCULIONIDAE: SCOLYTINAE) OF TANDOJAM**

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For present studies bark beetles were collected from bark of various trees of Tandojam. Further

examination and identification were carried out at insect systematic laboratory, department of Entomology, Sindh Agriculture University Tandojam. Collection was made through pooter, hand from various trees of Tandojam. In present study total 52 members of the superfamily Scolytini Latreille, 1804 were studied. During the course of identification of Scolytini 10 species were discovered under two tribes, Scolytini Latreille, 1804 with 08 species and Hylesinini Erichson, 1836 02 species. Scolytini Latreille, 1804 revealed the occurrence of 05 subtribes. 02 species under subtribe Cryphalina Lindermann, 1876 *Hypocryphalus mangiferae* (Stebbing, 1914) and *Cryphalus major* (Stebbing, 1903). 02 species under subtribe Scolytina Latreille, 1807; *Scolytus amygdali* Guérin Méneville, 1847 and *Scolytus rugulosus* (Müller, 1818). 01 species under subtribe Crypturgina LeConte, 1876; *Crypturgus pusillus* Gyllenhal 1813. 02 species under subtribe Ipina, Bedel 1888; *Pityogenes scitus* (Blandford 1893) and *Ips longifolia* (Stebbing 1909). 01 species under subtribe Pityophthorina, Eichhoff 1878; *Pityophthorus himalayensis* (Stebbing 1914). The other tribe Hylesinini Erichson, 1836 consisted of two species within subtribe Polygraphina Chapuis, 1869; *Polygraphus difficilis* Wood 1988 and *Carphoborus zhobi* Wood & Bright, 1992. 38th congress of zoology (International) February 27 – March 1, 2018 Department of Zoology, University of the Punjab Lahore.

COLLECTION AND IDENTIFICATION OF DELTOCEPHALINE LEAFHOPPERS OF SAKRAND

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For present studies specimens of deltocephaline leafhoppers were collected from various localities of Sakrand. Further examination and identification was carried out at insect systematic laboratory, department of Entomology, Sindh Agriculture University Tandojam. To identify the specimen up to the species level, keys for the region were collected from various publications. In present study total 140 members of the subfamily Deltocephalinae Fieber 1869 were studied. During the course of identification of Scolytini 10 species were discovered under six tribes, tribe Goniagnathini, Wagner 1951 revealed the occurrence of two species; *Goniagnathus quadripinnatus* Dash & Viraktamath, 2001 and *Goniagnathus guttulinervis* (Kirschbaum 1868); tribe Chiasmini, Distant 1908 with one species *Aconurella prolixa* (Lethierry, 1885); tribe Hecalini Distant, 1908 revealed with two species *Glossocratus breviceps* Morrison 1973 and *Hecalus porrectus* (Walker, 1858). Tribe Scaphytopiini Oman, 1943 was recovered with two species *Grammacephalus genoicus* Dlabola, 1984 and *Masiripius lugubris* (Distant, 1918), tribe Opsiini, Emeljanov 1962 with one record of species, *Opsius versicolor* (Distant, 1908); lastly tribe Stenometopiini, Baker 1923 was revealed with the occurrence of two species *Stirellus lahorensis* (Distant, 1918) and *Stirellus viridulus* (Pruthi, 1930).

MANAGEMENT OF APHIDS ON CANOLA (*BRASSICA NAPUS*) THROUGH CULTURAL PRACTICES

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Aphids are considered as major menace in limiting the crop yields. Keeping in view the losses caused by aphids, the present study was conducted to investigate comparative effect of different companion crops viz., barley, berseem, lucerne, wheat, maize, fennel seed, onion and garlic on aphid population in canola sown as border and intercrop. Results showed that minimum aphids in (46.00 and 49.50 aphids plant⁻¹) was observed in garlic sown as border and intercrop with canola and gave an enhanced grain yield of 1796.30 Kg ha⁻¹ and 2222.22 Kg ha⁻¹, respectively. Whereas canola plots with barley sown as border and intercrop showed significantly higher numbers of aphids (299.67 and 299.83 aphids plant⁻¹) with a lower grain yield of canola 602.42 Kg ha⁻¹ and 648.14 Kg ha⁻¹, respectively. While canola alone produced 740.75 and 787.02 Kg ha⁻¹ harboring 299.17 and 243.17 aphids plant⁻¹, respectively in border and intercrop experiments. It is suggested that the aphid infestation can be minimized by the use of companion crops like garlic, onion and fennel seeds.

SEASONAL MONITORING OF *BACTEROCERA* SPP. THROUGH INSTALLATION OF METHYL EUGENOL TRAPS AT DIFFERENT HEIGHTS IN CHIKU ORCHARD

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Study was conducted at Horticulture Garden Sindh Agriculture University Tando jam during the of chiku season of 2016-17. Methyl eugenol used 85%+ Sugar 10% Insecticide 5% used. The experiment was conducted to observe the *Bacterocera* species by hanging fruit flies traps at different heights on chiku orchard. The collection of data was started from 1st December, 2016 up to 31st March 2017. The experiment was consist on four treatments and five replication. Methyl eugenol traps were installed from ground surface up to 3m height. T1 ground surface, T2 1m height and T3 2m height, whereas; T4 traps were installed at 3m height. Experiment was designed Randomize Compete Block Design (RCBD). Two fruit fly species *Bactrocera zonata* and *B. dorsalis* were identified from the caught individuals. Overall mean number of *B. zonata* was 93.78, 149.6, 97.3, and 67.85 for T1, T2, T3, and T4, respectively. The highest trapped flies were observed in T2, whereas, the lowest mean number of flies were captured in T4. The results show that the highest numbers of *B. zonata* were captured in the month of December, 54.39 ± 22.56 . Whereas lowest population was measured in the month of January 4.75 ± 2.80 the mean number of recorded individuals in February and March remain 9.13 ± 2.69 , and 34.45 ± 3.51 respectively. The statistical analysis show that there was no significant difference was recorded among the traps regarding the number of fruit fly catches ($P > 0.05$.) Between all the treatments. Similarly, the highest mean number of *B. dorsalis* was recorded in December (28.96 ± 10.24) and the minimum density was recorded in January (0.83 ± 0.58). Treatment wise the highest number of *B. dorsalis* was recorded at T1 52.8 followed by T3 46.47 T2 46.5 and T4 34.61. The relation of temperature with *B. zonata* and *B. dorsalis* was positively correlated, whereas; there were negative relationship with humidity. On the basis of above mentioned results it is concluded that by installing methyl eugenol trapes at 1 m height more *B. zonata* flies can be captured as compare to other heights whereas to captured the maximum number of *B. dorsalis* ground surface is the best to trap more number of *B. dorsalis* 38th congress of zoology (International) February 27 – March 1, 2018 Department of Zoology, University of the Punjab Lahore.

SPECIES DIVERSITY OF TICKS PRESENT ON VARIOUS DOMESTICATED ANIMALS IN DISTRICT MULTAN

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Ticks and tick-borne diseases are the major problem for domesticated animals that cause extensive economic loss, mostly found in tropical and subtropical areas of the world. The main objective of present study was to determine the infestation load of various tick species on domesticated animals in Multan district. During this study, ticks were collected from different parts of the animal body such as ears, tail, neck, brisket, dewlap, back, hooves, testes and udder. A total of 1150 animals consisting of 400 buffaloes, 300 cows, 200 sheep and 250 goats, were sampled and more than 3300 specimens of ticks were collected. Tick infestation was recorded as 87.55%, 63.33%, 50.3% and 40% in buffaloes, cow, sheep and goats, respectively. Infestation rate was higher in sheep present in Shujabad as compared to those present in Multan and Jalalpur Pirwala. Maximum population was observed in June - July and minimum in October - November. Ticks prevalence was recorded more in female and young animals as compared to males and older ones. It was also scrutinized that indigenous breeds had more infestation than exotic and crossbreds. Collected specimens were preserved in 70 % Alcohol and brought to Ecological Lab, MNS-University of Agriculture, Multan, for identification. Preserved specimens were identified up to species level under the microscope with the help of identification key given by Walker *et al.*, (2014). The species identified were *Dermacentor marginatus*, *Rhipicephalus sanguineus* and *Hyalomma anatolicum*. The study can be significant in determining the risk of various tick borne diseases.

**DISTRIBUTION AND INCIDENCE OF GENUS *AIOLOPUS* (OEDIPODINAE:
ACRIDIAE: ORTHOPTERA) FROM DISTRICT DADU**

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The present study was conducted on distribution and incidence of *A. thalassinus* an important pest agriculture first time from various villages of District Dadu. A total 5362 specimens including various nymphal instars and adults were collected from different localities of District Dadu, during the year 2015-2016. A total of 5362 specimens of *Aiolopus* species were collected and their distribution and incidence in various villages are shown in Table # 01. The most dominant species was *A. thalassinus thalassinus* forming 69%, *A. thalassinus tamulus* 21% and *A. simulatrix* 10%.

**TRADITIONAL AND MODERN USES OF NATURAL HONEY IN HUMAN
DISEASES: A REVIEW**

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Honey is a natural by-product of flower nectar and the upper aero-digestive tract of the honey bees and it contains unique and distinct types of phenolic and flavonoid compounds of variable biological and clinical importance. It has been used both as food and medicine since ancient times. Human use of honey is traced to some 8000 years ago as depicted by Stone Age paintings. Pertaining to importance of honey in the traditional medicine some clinical and laboratory investigations by several research groups found its place in modern medicine. The review covers traditional and clinical applications of honey – such as, antioxidant, anti-inflammatory, antihyperlipidemic, cardioprotective and most well known effect of honey is antibacterial activity. Furthermore, the use of honey in treatment of eye disorders, gastrointestinal tract diseases, neurological disorders, fertility disorders and wound healing activity are also articulated. However, evaluation of the many potential biological and pharmacological activities of honey in the neurological disorders, cancer and related complications needs to be further explored.

EFFECT OF HEAVY METALS ON BIOLOGY OF LADY BIRD BEETLE

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Heavy metal contaminations are continuously increasing in the environment. Plants are the most common way to transfer heavy metals from soil to the insect body and to its systems. Heavy metals effect the physiology, morphology and genetics of the target insects. Chronic heavy metal effects include depression of growth, reproduction, development and hatching time, this also includes metabolic and respiratory changes. *Coccinella septempunctata* L. (Coccinellidae, Coleoptera) were collected and reared on artificial diet to get homogenous population for the experiment. Zinc Chloride (ZnCl₂) and Lead Nitrate {Pb(NO₃)₂} were used to study the effect of heavy metals on the adult weight, food consumption and mortality of *C. septempunctata* (L.). Different concentrations of ZnCl₂ and Pb(NO₃)₂ were applied with artificial diet. Data was recorded and analysed. Maximum mortality, food consumption and weight were observed at high concentration of Pb as compared to other treatments.

INVESTIGATION ON THE ORTHOPTERA FAUNA OF THAR ALONG WITH SOME NEW RECORDS OF ACRIDIDAE

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Grasshoppers and locusts caused damage to agricultural crops, pasture lands and forest. At the present extensive survey were carried out into different habitats of Thar. In this search, a fair numbers of Orthoptera species were collected but more prominent status was in favor of Acrididae. Following 4 -subfamilies viz: Gomphocerinae, Cyrtacanthacridinae, Tropidopolinae and Pyrgomorphinae were come in collection and occurrence of *Mesopsis laticornis* (Krauss, 1877), *Tropidopola longicornis* (Fieber, 1853) and *Atractomorpha acutipennis blanchardi* Bolivar, 1905 was constructed new records for Thar Sindh. Beside this, *Anacridium aegyptium* (Linnaeus, 1764), an Egyptian locust was also reported. It was also noticed that these species caused major damage to agriculture crops at such times the lower leaves are stripped to the midribs. In times of profile breeding, which are evidently quite frequent, all grass is eaten out and they then move into next field from the low-lying grasslands. As a further, aid to identification, illustration representing the different taxonomic characterizes are also presented. Acknowledgment: This research was supported by Higher Education Commission, Islamabad, Pakistan. (Project No 6737 SINDH\ NRPUR&D \ HEC \ 2015).

TAXONOMIC STUDIES ON SOME SPIDERS OF DISTRICT CHAKWAL

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Chakwal District is one of the important areas of Pakistan with respect to animal's diversity. The district contains different types of habitats which are very suitable for spiders to flourish. The study site was divided into nine different sites. Each selected sites were selected on the basis different habitats. Sampling was carried out using different methods and techniques and different time of day and night and summer and winter season. The Spiders were preserved and identified to family and genus level by observing different taxonomic characters using various keys in University of the Punjab. All spiders belonged to infra-order Araneomorph. 15 families and 28 genera were identified from collected spiders. Family Aranidae was dominant with respect to number of collected specimens followed by family Lycosidae and family Salticidae. Among the genera, Aranus has more numbers of specimens collected followed by Evipa, Lycosa, Oxyopes Marpisa and Plexippus.

STUDY ON THE VARIATION IN SPERMATHECA OF GENUS *AILOPUS* (ACRIDIDAE: ORTHOPTERA)

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Grasshoppers are those insects that have importance to carry out study to check the position of damage at agricultural crops and for to know their significance in insects taxonomy. During the present work orthopteran fauna in agricultural fields of Pakistan were investigated to collect the specimens of Genus *Aiolopus* to showing their morphology, spermatheca and distribution. The many specimens have been collected from different provinces of Pakistan during the field studies. Three species namely *Aiolopus thalassinus thalassinus*, Fabricius, *A.thalassinus tumulus*, Fabricius, and *A.simulatrix simulatrix*, Walker, of subfamily Oedipodinae was collected and viewed. Furthermore, the most dominant and widely distributed species was exposed *Aiolopus thalassinus thalassinus* their distribution has been reported and confirmed throughout the country. On other side

to update the knowledge of *Aiolopus* of Pakistan this work shows the great value of diagnostic features of phallic complex and epiphallus that has presumed as a tool for the sake of accurate identification. As well as this investigations may be proved to determine and control the pest incidences at agricultural fields. Besides this; some important characters of male and female genitalia have also been studied. All above studied species were recognized as serious pest of many valued crops in Pakistan. Besides this; some important characters of male and female genitalia have also been studied. All above studied species were recognized as severe pest of many valued crops in Pakistan. Present investigation has been carried out for the first time from this region.

FOOD PREFERENCE AND THEIR EFFECTS ON NYMPHAL STAGES OF GENUS *ACROTYLUS* FIEBER (OEDIPODINAE: ACRIDIDAE)

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Members of genus *Acrotylus* are major pest of arid and semi arid areas they also demonstrate wide diversity in their food preference. Nymphal stages of its two dominant species i.e *A. humbertinus* and *A. longipes subfasciatus* were studied in detail during the year 2014-2016. Nymphal stages were mostly herbivorous and graminivorous in nature and occupied different ecological niche. First of all food preference of various hopper stages of these species were investigated through regular field surveys and selected a set of five favorite food plants for laboratory rearing. It was concluded that total nymphal duration of *A. humbertinus* was significantly shortest on *Sorghum bicolor* with an average period of 25.56 ± 0.26 and 30.90 ± 0.56 days in male and female respectively whereas, prolonged duration was noted on *Abelmoschus esculentum* with an average of 38.28 ± 0.50 and 43.67 ± 0.29 days in male and female. In contrasting to this, total nymphal period of *A. longipes subfasciatus* was significantly fastest on mixed diet i.e 30.16 ± 0.57 and 30.24 ± 0.21 days in male and female while, *Saccharum bengalense* remained unsuitable food plant and prolonged the nymphal duration up to 45.62 ± 0.59 and 38.92 ± 0.52 days in male and female respectively. However, survivability of hopper stages was maximum on mixed diet; this knowledge will provide basic guideline to identify which plant is preferable by pest.

STUDY ON THE SUBFAMILY EYPREPOCNEMIDINAE (CAELIFERA: ORTHOPTERA) IN THAR DESERT

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An attempt was made to know the grasshopper abundance in Thar Desert. 4-localities i.e (Umerkot, Mithi, Islamkot and Nagarparkar) were selected and as a result 93 specimens were collected from August 2016 to September 2017, which were sorted out into single subfamily Eyprepocnemidinae, single tribe Eyprepocnemidini with 02 genera i.e *Heteracris* Walker, 1870 and *Eyprepocnemis* Fieber, 1853 pertaining to 02 species i.e *Heteracris littoralis* (Rambur, 1838) and *Eyprepocnemis alacris alacris* (Serville, 1838). During the present investigation significant morphological characters along with identification keys, photographs, illustrations and affected host plants were highlighted in order to make the identification easier for the future researchers. Further, it was also calculated that highest ratio of *H. littoralis* was 63.44% and *E. alacris alacris* with 36.55%. This research was financially supported by Higher Education Commission, Islamabad, Pakistan. (Project No. 6737 SINDH/ NRP/ R&D/ HEC/ 2015).

HYPOTHETICAL STUDY ON MIGRATORY BEHAVIOR OF SOLITORY *SCHISTOCERCA GREGARIA* FROM THAR DESERT

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Schistocerca gregaria prevail in Thar Desert from June to October each year which is consider as summer breeding area, while spring breeding area is Baluchistan. A hypothetical study is carried out to confirm the displacement of *S. gregaria* between Thar Desert and Baluchistan. Hypothesis was stated as "If the starting months of summer breeding areas (Thar Desert) encounter with large population of adults then it can be definitely said that adults could have been migrated over here from winter/spring breeding areas (Baluchistan) due to favorable condition". The hypothesis was measureable statistically with the parameter as number of adults and immature observed in fields of summer breeding areas. During field surveys it was observed that number of adult *S. gregaria* was very high in July, August, September and October, whereas incidence of immature in these months was low. From available evidence it could be concluded that adults might have migrated from Baluchistan to Thar Desert.

MORPHOLOGICAL AND BEHAVIORAL DESCRIPTION OF *OXYA HYL A HYL A* UNDER LABORATORY CONDITIONS

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Oxya hyla hyla a small hopper is considered the major pest of rice in Sindh. It causes million rupees damage annually. Apart from rice it also consumes sugarcane, wheat, herbs, shrubs and grasses in the field. At the present, some observations have been made on the life cycle of small rice grasshopper, *Oxya hyla* and found that the occurrence of instar period is greatly influenced by environmental factors and they also reported the various morphometries as well as different stages of instars and fecundity. During the present investigation it was found that the *Oxya hyla hyla* comprises on the sixth nymphal instars. There is significant difference was found amongst all the developmental stages of *Oxya hyla hyla*. In this manuscript all the morphological differences along with illustrations and measurement parameters were highlighted in order to determine the various stages of *Oxya hyla hyla*. Beside this, it was also noted that the measurement of various body parts was significantly different in various instars and sex ratio was in favor of females.

STUDY ON THE GENUS *CONOCEPHALUS* (CONOCEPHALINAE: TETTIGONIOIDEA) FROM SANGHAR DISTRICT

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The genus *Conocephalus* Thunberg, 1815 belongs to Conocephalinae. The grasshoppers belonging to this genus were common in the fields and are easily distinguished from the other grasshoppers found in Pakistan by their smaller size, slanting shaped head, long antennae and sword shaped ovipositor. A total of 52 samples were collected from the field and were sorted out into following 06 species i.e. *Conocephalus maculatus* (Le Guillou, 1841), *Euconocephalus pallidus* Redtenbacher, 1891, *E. incertus*, Walker, 1869, *E. indicus* (Redtenbacher, 1891), *E. mucro*, Do Haan, 1842 and *E. nasutus*, Thunberg, 1815. During this study morphological description of various species along with illustration was also provided. Beside this, it was noted that *E. incertus* feed on pine tree, rice, wheat, grass, sugarcane, edible podded, berry tree, common lawn and cultivated field. While, *E. pallidus* feed on sorghum, jowar, sunflower, sugarcane, sesbenia, gum *acacia* and *E.*

Mucro abundant in cultivated field and common lawn grass. Furthermore, *E. nasutus* found on sugarcane, berry tree, rice, edible podded, *prosopis* tree. *E. indicus* feed on sunflower, cultivated field, gum acacia tree, exotic gum *acacia* tree. *C. maculates* occurred in meadow grass, grass, rice, wheat, vinca/sadabahar, common lawn, sunflower, grape vine and cultivated field, the wide range of host plants show that they are severe pest of cultivated land.

DISTRIBUTION AND AGGRESSION BEHAVIOR OF FIELD CRICKETS (GRYLLIDAE: ORTHOPTERA) FROM DISTRICT NAUHAHRO FEROZE

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The Gryllidae occurring in a wide varieties of habitats i.e. trees, shrubs, herbs and grasses to swamp. The insects belonging to the Gryllidae fauna of Naushahro Feroze is very insufficiently known yet. Its population was maximum during the April, May and June. The average maximum and minimum temperatures during this period remain 41 °C to 24 °C respectively. Rainfall varies from year to year. During present study about 198 specimens were collected and sorted out into following 4 species i.e. *Acheta domesticus* (Linnaeus, 1758), *Gryllus (Gryllus) bimaculatus* Degeer, 1773, *Gryllodes sigillatus* (Walker, 1869) *Callogryllus ovilongus*. Sex was also identified on bases of wings, male have shorter, sturdier wings with rough underside surfaces known as file. A part from this, *Gryllidae* is omnivores, scavengers, and herbivores. During field survey it was observed that field male crickets have dominant, aggressive behavior to gain access to females and to protect their territory. Usually 06 aggression Levels i.e. Level 1; Pre-established dominance Level 2; Antennal fencing Level 3 & 4; Uni -& bi-lateral Mandible spreading Level 5; Mandible engagement and Level 6; Wrestling (all-out fight) are found in Gryllidae. Further, it was observed that most dominant species was *Acheta domesticus* with (45.45%) followed by *Gryllus (Gryllus) bimaculatus* with (27.77%) and least common species was noted *Callogryllus ovilongus*. However, from locality Moro we have collected the maximum number of specimens i.e. (118) while, from Kandiaro it were (80). This work will be first of its kind and will be of great help to institutions /agencies dealing with the pest control.

FEEDING BEHAVIOR OF CHROTOGONUS SPECIES (PYRGOMORPHIDAE: ORTHOPTERA) FROM DISTRICT KHAIRPUR MIR'S

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Species of genus *Chrotogonus* feed on almost any green vegetation and injurious to germinating crops and may inflict severe damage to cotton as well as wheat. These are polyphagous; feed on foliage and tender shoots. During the present study wide-ranging field survey was carried out to collect *Chrotogonus* from the various talukas of district Khairpur Mir's from March to November 2017. Collection was made from various field agriculture, semi mountainous and semi desert areas with vegetation of crops and grasses. On the basis of extensive survey it was noticed that species of *Chrotogonus* feed on the variety of crops coming in their way. Overall its 03 subspecies i.e. *C. (Chrotogonus) trachypterus trachypterus* (Blanchard, 1836), *C. (Chrotogonus) trachypterus robertsi* Kirby, 1914, and *C. (Chrotogonus) homalodemus homalodemus* (Blanchard, 1836) were found in the field. Beside this, it was also noted that *C. (Chrotogonus) trachypterus trachypterus* was dominant in the field compare to other species. Further, study is in progress it will rectify by molecular touch in future.

**SYSTEMATIC STUDY OF GENUS *CHROTOGONUS* (ORTHOPTERA: PYRGOMORPHIDAE)
FROM SUKKUR DIVISION, SINDH, PAKISTAN**

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During the year 2015-16 an extensive survey has been carried out in various areas of Sukkur division. In result 01 species and 02 subspecies of genus *Chrotogonus* were collected and identified as *Chrotogonus homalodemus* (Blanchard 1836), *Chrotogonus trachypterus trachypterus* (Blanchard 1836) and *Chrotogonus trachypterus robertsi* Kirby 1914. Beside this, description, identification keys, drawing lines and list of important host-plants were also studied. This significant study on this genus will be fruitful for the planning agencies in near future.

**COMPARATIVE STUDY ON THREE TRIBES OF OEDIPODINAE (ACRIDIDAE:
ORTHOPTERA) IN HYDERABAD DIVISION, SINDH, PAKISTAN**

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Grasshopper insect's fauna belonging to subfamily Oedipodinae of the family acrididae are of considerable economic importance. They pose constant threat to pastures and variety of crops in both irrigated and rain-feed areas of Pakistan. In extensive survey a total of 1949 specimen were collected i.e 1417 specimen instars and 532 adults during year 2017. All specimen belongs to three tribes Acrotilini, Epacromini and locustini and 08 species i.e *Acrotylus humbertianus* Saussure 1884 (26.16%), *A. longipes longipes* (Charpentier, 1845) (9.59%), *A. longipes subfasciatus* Bey-Bienko, 1948 (10.06%), *Aiolopus thalassinus tamulus* (Fabricius, 1781) (17.75%), *A. thalassinus thalassinus* (Fabricius, 1781) (7.75%), *Helithera aeolopoides* (Uvarov, 1922) (21.75%), *Locusta migratoria* (Linnaeus 1758) (1.48%) and *Oedaleus senegalensis* (Krauss, 1877) (5.43%). Furthermore, richness of species was calculated through Biodiversity Index $D = (n/N)^2$ and Simpson Index ($S=1-D$) for all species.

NEW RECORDS OF MANTODEA (DICTYOPTERA: MANTIDAE) FROM SINDH PAKISTAN

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The checklist of mantids first inventory species of the order Mantodea in the Sindh (Pakistan) is presented here with a new record from Pakistan i.e *Polyspilota aeruginosa* (Goeze, 1778) and *Deiphobe* Stal, 1877. The specimens were collected from Sanghar and its surrounding areas. 53 specimens were reported and distributed into single subfamily Mantinae, tribe Polyspilotini, genus *Polyspilota* and species *P. aeruginosa*. These all species are entirely different on the bases of morphometric characters and habits, but all are predatory in nature. It should be introduced as bio-control agent in future.

INVESTIGATION ON THE MIMICRY BEHAVIOR IN PHANEROPTERINAE SPECIES

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The bush crickets whose main character is to protect themselves from predators is camouflage in this behavior they retain the shape of their surrounding environment and objects which are present. The

Phaneropterinae species adopt the leaf color which is more helpful to hide themselves from predators. Mimicking behavior is defensive attribute found in bush crickets. Bush crickets do their most of the activities at the night time because they have less risk of being caught or spotted by other animals such as mammals or birds that are highly dependent on bush crickets for feeding purpose. The thick type of vegetation provides them a good shelter to defend themselves from enemies and at the risk of predation they remain motionless. Therefore, the thick vegetation is most favorable for the both adult male and female to vanish deep down to the base of vegetation where their approach reach to the two meters down in vegetation. Most of the orthopterans may face a large risk of predation at different heights in vegetation they remain in thick vegetation to protect themselves from aerial predators or higher above from the ground to evade from ground predators. Bush crickets are found in habitat of patches of vegetation at the different heights. Bush crickets are mostly found in clusters on the top of different altitudes of vegetation. This protective behavior has been studied for the first time.

STUDY ON THE MORPHOMETRIC CHARACTERISTICS OF OOTHECA IN MANTODEA FROM DISTRICT DADU, SINDH

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Mantodea is an order of fascinating group of predatory insects which commonly called as “Mantids” or “Praying mantis” and belong to the top predators of the arthropods community. In the result of extensive survey a total of 30 oothecae were collected during the different months of year i-e: 2016-2017. It was noticed that the eggs of a mantis are enclosed in a foamy pouch called an ootheca or egg sack. When the female produces the ootheca it is soft, but very quickly it will dry to become firm and tough. Every species of mantis has a different color, size and shape of ootheca. Oothecae vary in size and shape. Certain species of mantis will fashion large, round foamy ootheca; some will produce short, thick ribbon-like ootheca. The ootheca has an ovoid shape if it is observed dorsally. Its base, which is the area attached to the substrate (mainly the bark of trees), flat and its transversal section, has sub triangular. The front is tapered, flat and presents a plane process that has a cleavage in “U” in the center while, the back is rounded. The variation in the ootheca indicates the isolation of various species. This study will be very helpful to identify the different species of Mantids on ootheca basis so that rearing could be possible in future.

PREVALENCE OF ENSIFERAN SPECIES (ORTHEPTERA) FROM KHAIRPUR MIRS SINDH

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The Ensifera (long-horned) occurs in wide varieties of habitats i-e trees, shrubs, herbs and grasses, vegetation, forest and swamps. They cause considerable damage to all but particularly fruits were more effective by this. On its exact diversity, there is no work has been done yet it is therefore, felt necessary and present attempt has been made. 03 extensive surveys were carried out in district Khairpur Mir's Sindh, during the year 2016-2017 in different seasons. In result of survey a total of 152 specimens were collected and sorted out into 02 super families i.e. Tegtigonidea, and Grylloidea pertaining to 10 species viz: *Acheta domestica* (Linnaeus 1758), *Acheta meridionalis* Uvarov, 1921, *Phonarellus (Phanerellus) humeralis* Walker, 1871, *Gryllus bimaculatus* De-Geer, 1773, *Gryllus (Gryllus) quadrimaculatus apicalis* Bolivar, 1900, *Grylloides supplicans* Walker, 1859, *Grylloides sigillatus* Walker, 1869 of subfamily Gryllinae and 03 species namely *Trigonocorpha unicolor*, Stal, 1873, *Trigonocorpha angustata*, Uvarov, 1922 and *Conocephalus maculatus* (Le Guillou, 1841) of Conocephalinae were captured. Beside this, collection of Ensifera from different habitats and host plant was also presented which makes the material extremely valuable for scientific references.

OVIPOSITION BEHAVIOR OF ACRIDIDAE SPECIES (ACRIDOIDAE: ORTHOPTERA)**Muhammad Noman Bashir and Riffat Sultana***Department of Zoology, University of Sindh, Jamshoro*

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Grasshopper are considered as major pest of many valued crops in Sindh in order to control its population rate preliminary study has been done on its embryonic stages and during the present survey a total of 37 egg-pods were collected from different ecological zone of Sanghar in Sindh. After the examination, it was noticed that these egg-pods belong to 09 species. i.e. *Acrida exaltata*, *Truxalis fitzergaldi*, *Hieroglyphus perpolitia*, *Hieroglyphus oryzivorous*, *Hieroglyphus nigrorpletus*, *Oxya hyla hyla*, *Oxya velox*, *Oxyina bidentata* and *Oxya fuscovittata*. It was observed that all collected egg-pods have significant variation in size and shape and arrangement of egg. *Acrida* and *Truxalis* forming a single vertical series while the *Hieroglyphus* species is sub-cylindrical and *Oxya* species egg-pods were cylindrical and elongated in shape. It was also noted that egg-pods were full of eggs without any empty space. During oviposition, the female's abdomen may become completely buried in the earth, displacing excess material which I forced up into a mound around her during this process that the female's abdomen looks like an extraordinarily flexible and telescope organ.

ZONAL DISTRIBUTION OF ACRIDINAE (ACRIDIDAE: ORTHOPTERA) FROM MATIARI DISTRICT**Sadaf Soomro, Riffat Sultana and Barkat Ali Bughio***Department of Zoology, University of Sindh, Jamshoro*

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Acridinae hoppers are generally graminivorous in nature and pose constant threat to maize, chickpeas, beans and other legumes plants. During the present investigation 190 specimens were collected from the 03 tehsil i-e Matiari, Hala and Saeedabad of district Matiari. All grasshoppers of this sub-family are usually medium to large size, and tegmina pointed or obtusely rounded. Review of literature showed that, it was the more dominate sub-family and its occurrence reported all over the world. Its 140 genera have been reported yet while in the Pakistan its two genera i-e *Phlaeoba* Stal, 1861 and *Duroniella* Bolivar, 1908 was reported. Present study recommends that if more extensive survey would be done on regular base its numbers would be increased in future.

PREVALENCE OF DARKLING BEETLES (COLEOPTERA: TENEBRIONIDAE) IN SINDH**Farheen Deebea Soomro and Riffat Sultana***Department of Zoology, University of Sindh, Jamshoro*

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Family Tenebrionidae has great economic importance as it contain insect that are cosmopolitan in nature and most imperatively are associated with stored products. Darkling beetles are large group of insects that belong to the family Tenebrionidae. There are around 15000 species of darkling beetles that can be found in temperate and tropical regions around the world. The distribution of insect species is widely existed in Pakistan and causing economic damage to the number of plants and agricultural crops. Therefore, this study was conducted on the diversity status of subfamilies Tenebrionine and *Pimeliinae* of family Tenebrionidae. All the insects were collected through different methods like light trap and pitfall trap to observed their distribution and seasonal abundance. Most of the insect species are large in size and collected by hand directly from different part of Sindh during September 2016-2017. The main sources of collection for these species were soil surface of different farms and soil surface of jungle area and under the rock of mountain area specially lower Sindh of Pakistan and through the storages area. During this survey a total of 120 specimens of Tenebrionidae were

collected and sorted out into 03 species belong to 03 genera. However, it was notice that more dominate genera were *Tribolium* (Herbst, 1797) and *Trachyderma* (Forskål, 1775) in this region. These traps are monitor monthly and a total number of Tenebrionidae are calculated from all study sites. The present finding regarding prevalence of Tenebrionidae will be helpful in future to manage their population below economic threshold.

**SOME STUDIES OF THE SLANT FACED IMMATURE HOPPERS OF ACRIDINAE
(ACRIDIDAE: ORTHOPTERA) OF SINDH**

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The *Truxalis*, Fabricus, (1775) is one of the important genus of subfamily Acridinae contain quite number of species all around the world, causes serious damage to cash crops. At the present immature of 02 species i.e *Truxalis examia examia* Eichwald (1830) *Truxalis fitzgeraldi*, Drish (1951) were studied along with illustration which indicate that the major difference occurring in immature of this genus. This study is very helpful to isolate the five nymphal instars of this group.

DIVERSITY OF ACRIDIDAE SPECIES FROM THARPARKAR SINDH

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Acrididae encompasses the short-horned grasshoppers which are phytophagous insects and widely distributed throughout Tharparkar district. Acrididae species mostly found on common grown crops like Lady finger, Chili, Bajra and Cluster bean. Present survey a total 137 specimen were collected from different ecological regions of six talukas i.e. Chachro, Islamkot, Dhali, Nagarparkar, Diplo and Mithi of Tharparkar district during the month from May to September 2017. The collected material was categories into four subfamilies viz. Acridinae, Calliptaminae, Gomphocerinae and Hemiacridinae belonging to *Acrida exaltata* (Walker, 1859), *Acorypha glaucopsis* (Walker, 1870), *Hieroglyphus nigrerepletus* (Bolivar, 1912) and *Ochrilidia geniculata* (Bolivar, 1913) while, other specimens are in the process of identification. This study was financially supported by Higher Education Commission, Islamabad, Pakistan. (Project No. 6737 SINDH/ NRP/ R&D/ HEC/ 2015).

**PRELIMINARY OBSERVATION ON THE INCIDENCE OF STICK INSECTS
(PHASMATODEA: PHASMATIDAE) IN NARA DESERT KHAIRPUR, SINDH**

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Nara desert is characterized by high wind velocity, massive shifting and rolling of sand dunes; high diurnal variation of temperature; scanty rainfall; extreme solar radiation and high rate of evapo-transpiration. Nara Desert is rich with number of different species such as mammals, birds, reptiles and most dominant species of class insect. Phasmida are terrestrial, nocturnal, phytophagous insects found in nearly all temperate and tropical ecosystems. During the study very rare numbers of stick insect were collect from the Nara. During field it was noticed that stick insects are herbivorous, eating only plants and vegetation. They eat berries, vines, and leaves. They feed at night. Adult American Walking Sticks prefer to eat oak leaves. Stick insects mainly feed on leaves and other green plants, along with the odd berries or fruit. Due to their size, stick insects have numerous predators in their tree-surrounded environment, if they can be seen that is. Birds, small reptiles, and

rodents all feed on the stick insect if they are able to find one. This study work would be advantageous for future researchers who intend to study on taxonomy of stick insects from this region as well as economically valuable as pest of different vegetation.

RECORDS OF *MYRMARACHNE* MACLEAY, 1839 (ARANEAE, SALTICIDAE) FROM PAKISTAN WITH BARCODING ASPECTS

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Ant-mimicry has evolved in numerous families of spiders. Among jumping spiders, *Myrmarachne* is a genus of ant-like salticids. Although some mimics resemble ants very precisely in their morphology and behavior, others have only a superficial resemblance to ants. According to statistics of World Spider Catalog, *Myrmarachne* genus is based on 187 species. Only Five species have already been explored from Pakistan. In this study, 250 specimens were collected from different localities of Pakistan. GPS points, Micro and macro-habitat were recorded for actual and predicted species distribution. Vouchers specimens were identified to species level, photographed for general features and genitalia. DNA was extracted and barcoded for COI at ZFMK museum, Bonn, Germany. Additional sequences were retrieved from Gen Bank and BOLD to construct phylogenetic trees. In the data, four species, *Myrmarachne melanocephala* (♀), *Myrmarachne melanotarsa* (♂), *Myrmarachne tristis* (♂) and *Myrmarachne platyleoides* (♂) were new recorded from Pakistan. Furthermore, for all studied species, maximum intra-specific divergence was less than nearest neighbor distance. This suggested the reliability of DNA barcoding for spider's identification up to species level. MAXENT was used to predict the distribution range of these species in Pakistan. This will help in the collection of specimens for further studies.

POPULATION DYNAMICS OF EDAPHIC MICROARTHROPODS: A SIGNPOST OF SOIL QUALITY IN DIFFERENT LAND-USE TYPES

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Soil arthropods respond quickly to different land management practices and, hence, have been used as effective bioindicators of soil biological functioning and health. This study was aimed to determine the prevailing status of soil quality and health in different land-use types using edaphic microarthropods as bioindicators. Secondary objective was to assess the seasonal impact and influence of other environmental variables on the community structure of these microarthropods in each land-use type. Extensive soil sampling was conducted throughout the year from all tehsils of the district Sargodha (Punjab, Pakistan) and mites and springtails were extracted from these samples using Berlese-Tullgren method. Different land use types sampled in this study included grassland/ pastures, forest plantation, fallow land, sugarcane cropping, rice-wheat cropping, fallow citrus orchards and intercropped citrus orchards. According to results, there was a significant impact of seasons on the population abundance of soil microarthropods. During spring sampling (March-April 2017), highest population of collembola (springtails) and acari (predatory mites) were recorded in sugarcane fields followed by guava, fodder (berseem) and intercropped citrus and non-inter cropped citrus orchards. Very

few specimens were observed in natural land or uncultivated soils. In summer season (May-June 2017), same trend of collembola and mite population was observed but population abundance was quite low. In rainy season (July-August 2017), very low population of collembola was observed in all land-use types. Moreover, population abundance of collembola and mites were positively correlated with the total organic matter, carbon and nitrogen contents, soil moisture level and liter biomass present in different land-use types.

FIRST RECORD OF TWO SPECIES IN TWO GENERA OF CHILOCORINAE (COCCINELLIDAE: COLEOPTERA) FOR PAKISTAN

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Ladybird fauna of Gilgit-Baltistan region was explored and taxonomic studies confirmed occurrence of four species in four genera of subfamily Chilcorinae (Coleoptera: Coccinellidae). The identified species include *Chilocorus infernalis* Mulsant, 1853, *Exochomus nigromaculatus* (Goeze, 1777), *Exochomus trijunctus* (Kapur, 1969) and *Platynaspidium saundersi* Miyatake, 1961. *C. infernalis* and *E. nigromaculatus* are reported here as new records for Pakistan. Diagnostic characters of each species are given along with colour markings of elytra and detailed structure of genitalia. Remarks pertaining to taxonomic discussion, history, taxonomy status and regional record of distribution are given for each species.

A CONTRIBUTION TO THE KNOWLEDGE OF FEMALE GENITALIA AS A TAXONOMIC TOOL IN THE CLASSIFICATION OF TETTIGONIOIDEA

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A proportional study on the female genitalia was carried out in species of Tettigoniodea. An effort has been made to describe and illustrate the different structures i-e: Ovipositor, Cerci in female, Subgenital plate, Supra-anal plate in Tettigoniids with objective to determine their importance in order to make the identification of genera and species together with other generic characters more perfect and suitable. Genitalia structures chiefly female ovipositor and female cerci make it promising to put forward some suggestions about interrelation of families and subfamilies of Tettigoniodea more evidently than morphological characters.

GENUS ANAX (ODONATA, AESHNIDAE) FROM DISTRICT SWAT

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Family Aeshnidae is regarded as diverse group of Odonates with worldwide distribution. The members of this family comprises of 384 species (Tsuda, 2000). Aeshnidae are excellent flies with confluent compound eyes and a prominent ovipositor (female genital component). During the present study 251 specimens of dragonflies were collected from five localities of Swat district during year 2016-2017. The materials were sorted out into family Aeshnidae with single genus *Anax* with two species i-e *Anax imperator* and *Anax parthenope*.

TAXONOMY OF *CONOCEPHALUS* THUNBERG, 1815 (CONOCEPHALINAE: ORTHOPTERA) FROM DISTRICT MANSEHRA

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An extensive survey was carried out to collect long-horned grasshopper fauna from Mansehra district. The samples were taken into lab for further study. A 735 specimens were collected and sorted out into three species i-e : *Conocephalus* (Anisoptera) *fuscus* (Fabricius, 1793), *Conocephalus* (Anisoptera) *maculatus* (Le Guillou, 1841) and *Conocephalus* sp of genus *Conocephalus* Thunberg, 1815. The samples were observed under stereoscopic binocular microscope. Important taxonomic characters were noted down and line drawing of genitalia were made.

BIODIVERSITY OF WEEVILS (ENTIMINAE: CURCULIONIDAE: COLEOPTERA) FROM DISTRICT MANSEHRA KHYBER PAKHTUNKHWA PAKISTAN

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Present investigation was carried out during the year 2016-2017. The weevil's fauna belonging to subfamily Entiminae were collected through traditional sweep net, aspirator and on light traps from the various localities of District Mansehra. A total of 376 specimens were collected and sorted out into family Curculionidae Subfamily Entiminae and five genus i.e: *Compsus* Schönherr, 1823, *Ericydeus* Pascoe 1880, *Dermatodes* Schoenherr, 1840, *Rhytideres* Schoenherr, 1823, *Otiorhynchus* Germar, 1822 and five species i.e: *Compsus auricephalus* (Say, 1824), *Ericydeus lautus* (LeConte, 1856), *Dermatodes carinulatus* Motschulsky, 1863, *Rhytideres plicatus* (Olivier, 1790) and *Otiorhynchus meridionalis* Gyllenhal, 1834 respectively. The highest population of *C. auricephalus* was recorded with 27.92% followed by *D. carinulatus* with 23.67% while lowest population was found for the *E. lautus* with 7.18% *O. meridionalis* with 20.21% and *R. plicatus* with 21.01% respectively.

MORPHOLOGICAL STUDY OF DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA*) FROM DISTRICT MANSEHRA

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The surveys were carried out to capture the moth biodiversity particularly *Plutella xylostella* (Linnaeus, 1758) that belongs to family Plutellidae was collected from district Mansehra during the year 2016-2017. The specimens were taken into laboratory, killed and stretched on stretching board. The morphological characters were observed under stereoscopic binocular microscope. For genitalia study the samples were kept overnight into the desicator and were dissected out after 24hrs. The line drawings were drawn with the help of camera lucida connected with computer.

BIODIVERSITY OF ACRIDIDAE: ORTHOPTERA OF HAZARA DIVISION KPK PAKISTAN**Shoaib Ali¹, Waheed Ali Panhwar² Sardar Azhar Mehmood¹ and Shabir Ahmed¹**¹Department of Zoology, Hazara University, Mansehra KPK²Department of Zoology, Shah Abdul Latif University Khairpur Mir's Sindh

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An extensive survey was carried out during the year 2016-17 to find out Acridid fauna of Hazara Region. As a result of this work, a total of 1402 adult specimens were collected from different climatic zone of Hazara division during the year 2016-2017. Field sites included; agriculture land, forests, fruit orchards, grapevine, berry, shrubs, hilly, semi desert and desert areas, trees, shrubs, herbs and grasses. The collected material was sorted out into 73 species of Acrididae pertaining to 11 sub-families and 38 genera. Among them members of family Acridinae was found to be most abundant with 20.82%, followed by Oedipodinae, Gomphocerinae and Oxyinae with 17.61%, 17.47% and 14.40% respectively. Lowest population size was observed in Cyrtacanthacridinae and Tropicodinae with 3.06% and 2.49% respectively.

NATIVE SOLITARY BEES ARE EFFICIENT POLLINATORS FOR MUNGRA (*RAPHANUS SATIVUS*) SEED PRODUCTION**Muqadas Younas¹, Mudassar Ali¹, Shafqat Saeed¹, Asif Sajjad² and Mahreen Hanif¹**¹Department of Entomology, Muhammad Nawaz Shareef University of Agriculture, Multan²Department of Entomology, University College of Agriculture and Environmental Sciences, The Islamia University of Bhawalpur, Bahawalpur.

Bees have been documented as the most efficient pollinators for producing higher fruit and seed set in different vegetable crops. In order to determine the effectiveness of native bee species for the pollination success of mungra crop (*Raphanus sativus*), an experiment was conducted at the research farm of Muhammad Nawaz shareef University of Agriculture Multan, Pakistan. Pollination efficiency of most abundant pollinators was measured in terms of visitation rate, stay time (time spent per flower), stigma contact percentage along with single visit pod set percentage and number of seeds per pod. Visitation rate and stigma contact percentage was found significantly higher for *Xylocopa* sp. followed by the *A. dorsata* and *A. mellifera*. The single visit efficacy in terms of pod set percentage and number of seeds per pod revealed *Xylocopa* sp. as the best pollinator followed by the *A. dorsata* and *A. mellifera*. Moreover, pod set percentage and number of seeds per pod was significantly higher in open pollinated flowers compared to the self-pollinated flowers confirming the importance of insect pollination in this crop. Conserving these efficient native bee species may enhance production of this vegetable crop and provide higher return to the farmers in Punjab, Pakistan.

ROLE OF NATIVE INSECT POLLINATOR IN IMPROVING YIELD AND PHYSICOCHEMICAL PROPERTIES OF CUCUMBER (*CUCUMIS SATIVUS*)**Mahreen Hanif, Shafqat Saeed, Mudssar Ali, Unser Naeem Ullah and Muqadas Younas**

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Pollination is an essential but often neglected ecosystem service to mitigate crop yield gaps. Majority of cucurbits crops are monoecious hence pollination is the most important factor for enhancing the yield. Yield loss may occur up to 90 % in cucumber without insect pollination. Present studies was conducted at the research farm of Muhammad Nawaz Shareef University of agriculture Multan, during 2016 to evaluate the abundance and foraging behavior (stay time and visitation) of different insect pollinators in cucumber. Moreover, different pollination treatments (open, self and hand pollination) were also assessed for their influence on various physiological (size, diameter, weight, no. of seed per fruit and seed weight) and chemical (total soluble solids, titrable acidity and vitamin C) parameters of cucumber fruit. Among the insect pollinators visited, *Apis florea*

was recorded as the most abundant followed by the *Nomia* sp., and *E. aeneus*. Highest visitation rate was observed for *Nomia* sp. followed by the *A. florea* and *E. aeneus* while contrarily *E. aeneus* spent more time per flower (stay time) than the *A. florea* and *Nomia* sp. No significant differences were found between the hand pollinated and open pollinated treatment in terms of physiological and chemical parameters validating the effectiveness of insect pollinators for cucumber production. Therefore, conservation efforts for pollinator species should be adapted in this crop for getting higher yield and good return.

BUTTERFLY FAUNA FROM DISTRICT ABBOTTABAD

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Butterflies are the beautiful creatures belonging to order Lepidoptera. Suvery was carried out in the vicinity of district Abbottabad during the year 2016-2017. A total of 450 specimens were captured and identified into 16 species going to 2 different families i-e: Papilionidae and Pieridae respectively. Family Papilionidae occupies highest number of population with 75.55% and Pieridae 24.44%.

AGE RELATED FECUNDITY IN *CHRYSOPERLA CARNEA* (STEPHEN): IMPLICATION AND EFFECTIVENESS IN MASS REARING

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Egg-laying habits are distinctive in insect species. The stable age distribution during developmental time of adult affects the age-specific fecundity of *Chrysoperla* Spp. An experiment was conducted to investigate the age related effectiveness and potential of *Chrysoperla carnea* in mass rearing. Same age adults of *C. carnea* were obtained from culture reared under laboratory conditions at 25±2°C temperature with 65 % RH. These adults were kept for egg laying purpose on weekly basis. Results revealed that maximum eggs (103.5) of *C. carnea* female were laid in the 2nd week. While minimum eggs (6.5) were recorded in the 7th week of their emergence. Our results indicated that egg potential decreases as age increase of *C. carnea*. The findings of this study have significant role in mass production of *C. carnea* under laboratory conditions.

BIODIVERSITY OF CULICIDAE IN DISTRICT BAGH AZAD KASHMIR

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In the present study the Mosquito's fauna of the district Bagh was explored; samples were collected from different locations of the study area, extending from May to October, 2017. Specimens were collected by using hand net and mouth aspirator; collected specimens were pinned up and stored in the entomological box. Specimens were identified by using the taxonomic keys. A total of 1036 specimens were collected from the study area, belonging to the family Culicidae, order Diptera, two subfamilies anophelinae and culicinae and four genera Anopheles, Culex, Aedes and Armigeres and eleven species; *Anopheles bariansis*; *Culex pipiens*, *Culex epidesmus*, *Culex pseudovishni*, *Culex fuscocephala*, *Culex pipiens fatigans*, *Culex vishnui*, *Culex barraudi*;

Aedes agiptie, *Aedes micropterus* and *Armigeres subalbatus* most abundant species was *Culex pipiens*. This study will provide the base line data to the future researchers and better epidemiological understanding.

COMPARATIVE MORPHOLOGY OF EXTERNAL GENITALIA OF SOME DRAGONFLIES (ANISOPTERA: ODONATA)

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Dragonflies (Anisoptera: Odonata) are predatory insects possessing important role in controlling populations of many harmful insects like mosquitoes, flies, biting midges, aphids and whiteflies etc. They act also as significant indicator of water quality. In Pakistan, 68 species from 5 families of Anisoptera have been reported so far. In class Hexapoda, these creatures have unique feature of having their external male genitalia on second abdominal sternum. Morphology of genitalia is an important taxonomic attribute which help to differentiate species from one another. Keeping the importance of external genitalia in view, the study was planned. In this regard, drawings of external male and female genitalia of some species of dragonflies were made with the help of camera lucida under the microscope. A total of 11 species were included in the current study, viz., *Crocothemis erythraea*, *C. servilia*, *Pantala flavescens*, *Diplacodes lefebvrei*, *D. trivialis*, *Selysiothemis nigra*, *Brachythemis contaminata*, *Orthetrum sabina*, *Tramea basilaris*, *Trithemis pallidinervis* and *Acisoma panorpoides panorpoides*. Shape and structure of genitalia will help in identification of species especially closely resembled such as *Crocothemis erythraea*, and *C. servilia* and many others.

RESPONSE OF ABIOTIC FACTORS AND PHISICO-MORPHIC CHARACTER ON POPULATION FLUCTUATION OF JASSID, *AMRASCA BIGUTTELLA BIGUTTELLA* (HOMOPTERA : JASSIDAE) ON BOTH TRADITIONAL AND TRANSGENIC CULTIVARS OF COTTON.

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Jassid, *Amrasca devastans* (Dist) is serious sucking insect of cotton (*Gossypium hirsutum* L.). Different management strategies including chemical control have been adopted to control the pest. The mainly problems of chemical control including harmful effects on nontarget insect, insecticides resistance in the pest, environment degradation and host plant resistance is considered to be effective against jassid. The present study was carried out in order to determine the comparative resistance or susceptibility in AA-703, AA-802, SITARA-08, FH-113, CRSM-07 of transgenic cultivars and CIM-496, FH-1000, FH-941, FH-942, FH-901, of traditional cultivars of cotton against jassid. The data on jassid population per leaf obtained from cultivars in 2014-2015 various dates of observations were correlated with weather factors such as temperature, relative humidity and rainfall. The selected cotton cultivars (Transgenic and traditional) were sown with three replications by using randomized complete Block Design (RCBD). Correlation among the weather factors and jassid population showed that temperature were positively correlated with jassid population while relative humidity and rainfall were negatively correlated. Temperature has significant positive effects on all cultivars. Correlation between jassid population and phisico-morphic plant characters revealed that jassid population showed negative and non-significant correlation with hair density, gossypol glands length of hair and showed negative and significant correlation with thickness of leaf lamina.

REDESCRIPTION OF GENUS *COLOTIS* (HUBNER 1819) WITH REFERENCE TO THEIR MALE AND FEMALE GENITALIA

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Genus *Colotis* belongs to subfamily Coliadinae, family Pieridae and order Lepidoptera. This group of butterflies is small to medium sized, found all over the world wherever the host plants are available. They are very beautiful and attractive butterflies due to their different colour such as red, brown, blue and black. Presently, four species of genus *Colotis* were identified namely *Colotis amata* (Fabricius), *Colotis danae* (Fabricius), *Colotis protractus* (Butler) and *Colotis vestalis* (Butler) from different localities of Sindh, Pakistan. These species were described on the basis of morphological characters such as mouthparts, thorax and abdomen along and male and female genitalia illustrated for the confirmation of the species. This is first time record from Sindh, Pakistan.

STUDIES OF BIONOMICS OF MOSQUITO LARVAE IN MULTAN

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Current study was designed to evaluate the environmental characteristics and habitats governing the presence of larvae of different mosquito species. For the purpose, surveys were conducted to observe the larval population of mosquito species on different types of water qualities in Multan. Larvae of mosquitoes were collected from urban and rural areas of Multan. Biotic (Living aquatic organism etc.) and abiotic factors (TDS, R.H., pH and Temperature etc.) were recorded during survey. Collected samples were taken to Ecology Lab and after boiling preserved in 75% ethanol. The preserved larvae were then identified with the help of microscope and available taxonomic keys, up to species level. Population fluctuation was recorded on fortnightly basis and data were analyzed through correlation between biotic and abiotic factors. Only one dengue vector species out of two viz., *Aedes aegypti*, was recorded during the study from the area under work. Room coolers were observed as preferred habitat to proliferate for *Ae. Aegypti*. Among other collected species, *Culex quinquefasciatus*, *Cx. Bitaniorhynchus* and *Anopheles stephensi* were among the common species. This study will be helpful in determining the temporal and spatial distribution of different mosquito species in Multan, which can further be incorporated in surveillance programs and preventive measures of vector borne diseases like Malaria, Dengue, Zika etc. In South Punjab, rainfall is not abundant, so the mosquito breeding can be minimized by managing the water sources and mechanical elimination of potential breeding sites.

NEW RECORDS OF CHEWING LICE (PHTHIRAPTERA: INSECTA) ON DUCKS (AVES: ANSERIFORMES: ANATIDAE) FROM HAMAL LAKE IN SINDH, PAKISTAN

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Chewing lice (Phthiraptera) are parasitic insects, found on birds and mammals. During present survey, a variety of 25 ducks species have been examined for their chewing lice from Hamal Lake at District Qambar Shadad Kot during March 2017 to Nov 2017. In all 25 birds, belonging to five species, Common pochard

(*Aythya ferina*), Tufted duck (*Aythya fuligula*), White-eyed pochard (*Aythya nyroca*), Common teal (*Anas crecca*) and Garganey (*Anas querquedula*); 21 ducks were infested with chewing lice of both suborders Amblycera and Ischnocera included family Menoponidae and Philopteridae respectively were reported from Hamal Lake in District Qamabr Shadadkot. It was the first chewing lice survey that has been carried out in the region, which reported two species of family Menoponidae are *Trinoton querquedulae* (L.1758) and *Holomenopon leucoxanthum* (Burmeister, 1838) and three species of family Philopteridae were *Anaticola crassicornis* (Scopoli, 1763), *Anaticola mergiserrati* (De Gear, 1778) and *Anatoecous icteroides* (Nitzsch, 1818). All were new records from Hamal Lake in Sindh, Pakistan.

GUILD COMPOSITION OF ORDER DIPTERA, NEUROPTERA AND ORTHOPTERA IN DIFFERENT ECOSYSTEMS

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This study was conducted to record the “Guild composition of order Diptera, Neuroptera and Orthoptera in different ecosystems” during the session 2015-2016 under the ecological conditions of district Faisalabad. Maximum population was recorded from Wetland 37.69% (N = 940), followed by Agro-ecosystem 37.65% (N = 939) and least population was recorded from Forest area 24.66% (N = 615). In case of Agroecosystem, maximum biomass was recorded during 12th sampling (2.08±0.69) at 31°C and 51% humidity, and least biomass was recorded during 9th sampling (0.35±0.54) at 24°C and 30% humidity. From Wetland, diversity (H') was recorded highest (2.2350) as compared to Forest and Agroecosystem (2.1538) and (2.2348), respectively. Highest evenness value was again recorded from Wetland and Agroecosystem (0.0790) and least from Forest (0.0551). Dominance was recorded highest from Wetland and Agroecosystem (1.0790) and least from Forest (1.0551). However, richness (R) was recorded maximum in Wetland (12.5661) and least from Forest and Agroecosystem (8.4510) and (8.1922), respectively. Analysis of Variance (ANOVA) was made, after completing the analysis and it was observed that population mean of recorded taxa among different ecosystems (agro-ecosystem, wetland and forest area) showed non-significance results (F=0.06; P=0.9949). t-test showed that in overall strength, order Diptera, Neuroptera and Orthoptera were existed non-significantly in Agro-ecosystem-Forest (t-value= 0.71; P-value= 0.5530), Agroecosystem-Wetland (t-value = -0.06; P-value= 0.9595) and Wetland-Forest (t-value= 0.68; P-value= 0.5648). Linear regression showed that structural community as well as taxa composition were differing significantly among Agroecosystem-Forest (F= 0.71; P ≤ 0.1133), Agroecosystem-Wetland (F = -0.06; P ≤ 0.0051) and Wetland-Forest (F= 0.68; P ≤ 0.1183). From Forest area, predator, scavenger and parasite ratio was recorded up to 4.39%, 8.94% and 14.47%, respectively. While pest ratio exceeding up to 88.71% in Agro-ecosystem. From Forest area, number of specimens were recorded pollinator 1.62% (N = 10) and 34.6% (N=326) from Wetland, while, from Agroecosystem excessive population 2.34% (N = 22) was recorded.

EFFECT OF DIFFERENT PROTEIN BASED DIETS ON THE BIOLOGY OF BACTROCEA CUCURBITAE UNDER LAB CONDITIONS

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Proteinous diets have great influence on the biological parameters of tephertid flies. Four different kinds of protein based diets including Troula yeast, Brewer yeast, Protein hydrolysate and casein were tested as an

adult diet of *Bactrocera cucurbitae* under lab conditions. Results revealed that significantly higher female (~71 days) and male (65.05 days) longevity was recorded when *B. cucurbitae* were supplied with protein hydrolysate and lowest female (64.25 days) and male (53.25 days) longevity was recorded in casein. Moreover, higher fecundity (21.25/day/female) and higher fertility (92.75%) was recorded when the adults were supplied with protein hydrolysate and lowest in Troula yeast (14.25 and 81.75), respectively. However reduced larval period (5.88 days) was recorded when adults of *B. cucurbitae* were provided with protein hydrolysate and increased (7.18 days) in casein. Reduced pupal period (6.5 days) was observed in Troula yeast and increased (8.75 days) on casein. Higher female were yielded when the *B. cucurbitae* were given with protein hydrolysate and higher males on casein. The results of the studies would be helpful for mass rearing and IPM of *B. cucurbitae* on a variety of cucurbit crops.

EXPLORING THE LADYBIRD BEETLE OF (COLEOPTERA: COCCINILIDAE) OF DISTRICT MARDAN

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Ladybird beetles are universal predator and play very important role in biological control. However the ladybird beetle fauna of Pakistan is not extensively explored area wise. The present study was conducted to explore the ladybird beetle fauna of district Mardan, KP, Pakistan. The species were collected from twelve different localities of Mardan district, namely Sawal Dher, Palo Dheri, Charguli, Machi, Shabaz Ghara, Rustam, Toot Kali, Jamal Ghari, Katlang, Takhtbhi, Husai and Surkh Dheri. Collection surveys were conducted in the active season during 2017. During the study the collected specimens were identified that there are eight species of ladybird beetles under eight genera and one sub family Coccinellinae were collected. These eight species are *Harmonia dimidiata*, *Cheilomenes sexmaculata*, *Coccinellaseptempunctata*, *Coccinellatransversalis*, *Caelophorabisseltata*, *Menochilussexmaculatus*, *Propyleadissecta* and *Coccinellaundecimpunctata*. The numerical data of the species reveals that *Harmoniadimidiata*, *Menochilussexmaculatus*, *Coccinellaseptempunctata* are highly abundant and were collected from all localities. In this research work we study the diagnostic characters of each species, colored plates of adult specimens, taxonomic distribution, history, taxon status, seasonal occurrence, host and regional record are given for each species.

POTENTIAL ALTERATIONS IN THE DISTRIBUTION OF FOLIAGE INSECTS IN RESPONSE TO DAY RHYTHM IN OKRA FIELDS

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The present study was conducted to accord the "Potential alteration in the distribution of foliage insects' form in response to day rhythm in Okra field" during the session 2015-2016 under the ecological conditions of district Faisalabad. Overall 3434 specimens were recorded from Okra fields whole, maximum population was recorded at Morning 36.60% (N = 1257) and least population was recorded at Noon 30.63% (N = 1052). In case of Morning, maximum biomass was recorded during 3rd sampling (3.01 ± 1.08) at 29°C and 62% humidity, and least biomass was recorded during 4th sampling (0.61 ± 0.47) at 24°C and 73% humidity). In morning fields, from total of 7 recorded orders, 7 orders were recorded, and relative abundance was recorded maximum for order Diptera (Butterflies) 29.83% (N = 375), followed by Hemiptera (bugs) 22.99% (N = 289), Araneae (spiders) 13.84% (N = 174), Coleoptera (Beetles) 12.57% (N = 158), Hymenoptera (ants) 11.30% (N = 142), Orthoptera (Crickets) 5.81% (N = 73) and Lepidoptera (Flies) 3.66% (N = 46). In case of Noon all orders were

recorded and relative abundance was recorded extraordinary for order Hemiptera (bugs) 30.13% (N = 317), followed by Diptera (Butterflies) 29.66% (N = 312), Orthoptera (Crickets) 4.75% (N = 50) and Lepidoptera (Flies) 2.47% (N = 26). In case of Evening all orders were recorded, and relative abundance was recorded extraordinary for order Diptera (Butterflies) 32.09% (N=361), followed by Hemiptera (bugs) 21.16% (N=238), Hymenoptera (ants) 15.38% (N=173), Coleoptera (Beetles) 11.11% (N=125), Aranae (spiders) 97.29% (N=82), Orthoptera (Crickets) 6.76% (N=76) and Lepidoptera (Flies) 6.22% (N=70). Analysis of Variance (ANOVA) showed non-significance results for comparative occurrence (F=0.12; P=0.8906). Linear regression confirmed that structural community as well as taxa composition were differing significantly among all times (F=37.14; P ≤ 0.0017).

EXPLORING THE DIVERSITY OF VESPID SPECIES OF DISTRICT SWAT, PAKISTAN

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Members of family Vespidae are winged insects having fascinating colouration about 5000 species throughout the planet. However, tropical area are much diverse. Majority of wasps are carnivorous. Female can sting and used for paralyzing the prey to feed upon. The aim of the current study was to find out wasp diversity in Kabal Swat. Although wasp species has been studied in most areas of the world but wasp data for region such as District Swat is lacking. During entomological survey from September 2016 to October 2017 about 2600 specimens were collected from different villages of Tehsil Kabal District Swat. Collected specimens comprises of 10 species of three subfamilies belong to four genera. These Subfamilies are Polistinae, Vespinae, Eumeninae. *Vespa orientalis* Linnaeus, (1771), *Vespa tropica* (Bequaert, 1933), *Vespa velutina* (Lepeletier, 1836) from one genus of subfamily Vespinae. However, subfamily Polistinae is represented by such as *Polistes rothneyi carletoni* Van der Vecht, (1968), *Polistes (Gyrostoma) wattii* Cameron, (1900), *Polistes flavus*, *Polistes hebraeus fabricius*, *Polistes (Polistella) stigma tamulus* (Fabricius, 1798). *Delta dimidiatipenne* de Saussure, (1852) and *Rhynchium quinquevittatum* belong to subfamily Eumeninae. Beside this Morphometry of wasp was also recorded.

FIRST RECORD OF GENUS *ACTORNITHOPHILUS* FERRIS, 1916 (PHTHIRAPTERA: AMBLYCERA: MENOPONIDAE) FROM SINDH, PAKISTAN

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In the continuation of chewing lice faunal study in Sindh, Pakistan, the present study has been carried out for aquatic and semi aquatic birds. Presently, the chewing lice species of the genus *Actornithophilus* Ferris, 1916 have been recovered from the birds of order Charadriiformes. 05 birds of black-winged Stilt (*Himantopus himantopus* (L.)) of family Recurvirostridae and 07 birds of wood sandpiper (*Tringa glareola* (L.)) of family Scolopacidae were examined for their chewing lice. Among 12 birds, three species of chewing lice of genus *Actornithophilus* have been collected, including *A. himantopi* Blagov. 1981 and *A. lyallpurensis* Ansari, 1955 from black-winged stilt whereas *A. totani* (Schränk, 1803) and *Actornithophilus* species from green sandpiper were recovered. All species of the genus have been recorded for the first time from Sindh, however *A. lyallpurensis* was previously reported from Faisalabad (Punjab) and second time from Sindh; making a new addition in the chewing lice fauna of Sindh, Pakistan.

**NEW RECORD OF TIGER BEETLE SPECIES *ANTHIA SEXGUTTATA SEXGUTTATA*
(COLEOPTERAN, CARABIDEA) FROM JAMSHORO, SINDH**

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Anthia sexguttata sexguttata (Fabricius 1775) commonly known as tiger beetle or ground beetle belong to the family Carabidea. During the present study. Single pair was reported from Jamshoro. It morphometric observation is under: length of head =1.8mm, length of pronotum =0.8mm, length of antenna =1.5mm, length of abdomen=2.1mm and total body length=4mm for male, while in female it was length of head=1.8mm, length of pronotum=0.9mm, length of antenna=1.6mm, length of abdomen=2.3mm and length of total body=4.1mm. Presence of six spots on 02 on thoracic region and 04 on the elytra is key character of tiger beetle due to this character it is commonly known as six spot ground beetle. Female is larger in size than male. They are carnivorous in nature. From Sindh no work has been done on the morphological character of tiger beetle, present study is the starting point to introduction of this beetle from Jamshoro.

**STUDIES ON DISTRIBUTION OF DARGON AND DAMSELFLIES ODNATES
FROM DISTRICT JAMSHORO**

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During the present investigation 350 specimens were collected from the different areas of District Jamshoro. Material sort out into 6 species of dragonflies and damselflies present in this areas namely *Aethriamanta brevipennis* (Rambur 1842), *orthetrum Sabina* (Durry 1775), *Bradinopyga germinate* (Rambur 1842), *Ishnura elegans* (Vandar linden, 1820), *Nehalennia gracilis* Morse. 1895, *Ishnura verticalis* (Say, 1839). It is expected that more extensive survey will enhance it further wealth.

**MOLECULAR CHARACTERIZATION AND PHYLOGENETICS OF FAMILY GOMPHIDAE
(ODONATA: ANISOPTERA) OF HAZAR REGION PAKISTAN**

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Current study was conducted on molecular characterization of Gomphid dragonflies collected from Hazara region of Pakistan. A total 345 specimens were collected and identified in to 6 genera covering 9 species. Molecular phylogenetic relationships among members of the family Gomphidae were examined using 735 bp of mitochondrial COI gene and 416 bp of 16S ribosomal RNA gene sequences. Phylogenies of the analyzed taxa were elaborated with maximum likelihood, maximum parsimony and Bayesian analysis. The COI gene and 16S rRNA gene separate and combined COI+16S data sets revealed evolutionary relationship within family Gomphidae at the species and genera level. A total of 1543 nucleotide sequences and 46170 genetic characters/position were used for the COI, 16S and combined COI+16S data set. Mean Pairwise Distances (MPD) of each species were ranged from 0.00 to 37.10%. Evolutionary rate differences among two categories Gamma distribution and Invariant (+G+I) were recorded as 0.06 and 1.20 substitutions per site. DNA based identification using COI, 16S and combined COI+16S data set analyses showed genetic similarities having bootstrap values MLB=70-100%, MPB= 52-100% and BPP=0.75-1%. The analysis of the combined COI+16S

data set yielded trees with overall stronger bootstrap support than analyses of either gene alone. Likelihood, Parsimony and Bayesian analyses of the combined COI+16S data set produced well-resolved phylogenetic status of recorded species. The phylogenetic significance of these results is discussed and experimental approaches that would advance our understanding of Gomphidae dragonflies diversity based on molecular study.

**A NEW SPECIES OF GENUS *ACHETA* F., *A. KARACHIENSIS* (ORTHOPTERA: GRILLIDAE)
WITH SPECIAL REFERENCE TO ITS TEGMEN AND MALE GENITALIA FROM
KARACHI, SINDH, PAKISTAN**

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In the present work a new species of *Acheta* F. is described from Karachi, Pakistan with special reference to its tegmen and male genitalia. Previously, the cosmopolitan species, the common house cricket *Acheta domestica* (L.) and recently known species *A. khanpurensis* Khan and Ahmad 2016 were known from Pakistan. In the recent research external morphological characters of the new species are compared with that of the *Acheta* species found in Pakistan with its allies from Asia. Kamaluddin *et al.* (2001) and Khan and Kamaluddin (2006) described the external morphology of different species of Ensiferan fauna from Pakistan and also discussed their cladistic relationship. Ashraf (1978) attempted the worked on the comparative external morphology of the genus *Gryllus* (L.) and *Acheta* (F.). Specimens of *Acheta karachiensis* were collected from Karachi, Pakistan, at night with sweeping and hand picking techniques. After preservation, the entire specimens were boiled, for few minutes to soften for the study of tegmen and genital components. After washing, the right tegmen was detached from the body and placed on a slide. The tegmen was covered with cover slip to take the photograph, whereas the genital components were dissected out under Nikon SMZ 800 Binocular. in the same media. The photographs of tegmen and male genitalia were taken by using Nikon Cool Pix 5400 digital camera having placed them under Nikon SMZ 800 Binocular. After completion the work, the genitalia was preserved in vial with glycerin.

PRODUCTION OF HONEYBEES AND HONEY FROM DISTRICTS KARAK AND KOHAT

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Honey bees are considered social insects living in the form of colonies or apiaries. Honey bees are present in all parts of the world except extreme Polar Regions. Beekeeping is still carried out in different parts of the world and honey can still be a life saving food for prehistoric people in time of food calamity. The current study was conducted to find out the rearing of honey bees and honey production in different bee farms of districts Kohat and Karak during 2011-2014. Districts Kohat and Karak are ideal localities for the rearing of honey bees and honey production, having different honey bee Flora in which *Zizipus jayuba* (Beer) and *Acacia modesta* (Paulisa) are present in abundance throughout the year. Terewal Banda, Darmalok, Billi Tang and Siyab have the maximum number of apiaries during 2011-2014. In the same year, Gumbut, Bharathi Banda and KDA Kohat have the minimum number of apiaries were observed from district Kohat. Siyab, Darmalok, Billi Tang and Shakardara produce the highest honey production while Gumbut, Bharati Banda, Shakardara and KDA Kohat have the lowest. In district Karak the highest number of apiaries was identified in Amberi Kala, Teri and Bhader Khel. Teri is considered as a rich representative area for the production of apiaries. Banda Daud Shah, Saber Abad and Ahmad Abad having the lowest number of apiaries. Among these areas Banda Daud Shah is considered as a poor zone for the rearing of honey bees. Teri and Bhader Khel produce the

maximum honey production while Warana, Saber Abad and Banda Daud Shah Produce have the lowest. Overall highest honey production (2504.6) kg was found in Terawal Banda while lowest (144.2) kg was in Gumbat whereas highest per apiaries production (5.20) was also found in Terawal Banda from district Kohat. The highest productions (2890.7) kg was in Latamber while lowest (105.6) kg was in Warana whereas highest per apiaries productions (5.3) were in Ahmad Abad from district Karak. Interestingly the highest production (2890.7) kg was observed in district Karak compared to district Kohat during 2011-2014. By introducing the modern apicultural techniques the average honey and rearing of honey bees can increase in the mentioned areas. Wasps and mites can cause a great loss to the honey bees whereas some other diseases are also observed. Ecological factors including dust, rain, temperature, water and pollution affects the rearing of apiaries and honey production from districts Karak and Kohat.

ABUNDANCE OF SCARAB BEETLES (COLEOPTERA: SCARABAEIDAE) IN HYDERABAD AND ITS ADJOINING AREAS, SINDH

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Scarab beetles belong to order Coleoptera (largest order of Insecta) and family Scarabaeidae with approximately 30,000 species of beetles throughout the world, scarab beetles are phytophagous and coprophagous. Adults of many scarab beetles are noticeable due to their relatively large size, bright colors, often elaborate ornamentation, and interesting life histories. Weekly observations were made and the specimens were collected by multiple methods (mercury light trap, pitfall trap and hand picking) result showed that the abundance is correlated with lunar cycle, temperature and physical factors.

ANTIOXIDANT POTENTIAL OF FOUR HEARTWOOD EXTRACTIVES AND THEIR IMPACT ON MID GUT ENZYME ACTIVITIES IN *HETEROTERMES INDICOLA* (ISOPTERA: RHINOTERMITIDAE)

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Heterotermes indicola is a species of subterranean termites that are destructive pests of wood and wood products in Pakistan. This study evaluated the antioxidant and antienzyme potential of heartwood extractives against *Heterotermes indicola*. Heartwood extractives of four durable wood species, *Tectona grandis*, *Dalbergia sissoo*, *Cedrus deodara* and *Pinus roxburghii* were removed from wood shavings via soxhlet extraction with an ethanol: toluene solvent system. The antioxidant potential of the extractive compounds was determined using the DPPH radical scavenging test. Results showed maximum antioxidant activity for extractives of *D. sissoo*, which had the lowest IC₅₀ (concentration where 50% inhibition of DPPH radical is obtained) at 28.83 µg ml⁻¹ among the heartwood extractives. This antioxidant activity, however, was not concentration dependent as was observed in the other heartwood extractives tested. At the maximum test concentration, *T. grandis* showed the highest percent inhibition (89.7%) but this inhibition was lower compared to the positive control antioxidant compounds BHT and quercetin. When termites were fed filter paper treated with IC₅₀s of the extractives and control compounds, Glutathione S-transferase activity in the guts of *H. indicola* workers was significantly reduced by *T. grandis* and *D. sissoo* extractives. Similarly, esterases activity were reduced more by *P. roxburghii* extractives compared to control antioxidant treatments and other tested extractives. However, none of the extractives examined significantly reduced the activity of catalase enzymes in *H. indicola* compared to treatments with antioxidant control compounds.

**THE STRUCTURE OF THE EGGS OF SEVEN SPECIES OF WESTERN EUROPEAN
TETTIGONIIDAE (ORTHOPTERA) AS SEEN UNDER SCANNING
ELECTRON MICROSCOPE (SEM)**

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The Scanning Electron microscopic study was carried out on inner and outer layers of chorion, respiratory system between these layers and external surface of the eggs of seven species of Tettigoniidae from Western Europe. The following species egg were studied: Phaneropterinae: *Leptophyes laticauda* (Friv), *Isophya* sp; Decticinae: *Antaxius sorrezensis* (Marquet), *Pholidopter aptera*(F), *P. littoralis* (Fieb.), *Pholidoptera* sp. Ephippigerinae: *Ephippigerephippiger* (Fiebig). Eggs of the species were obtained from the captive female. The eggs were opened and the contents removed under water. The shells were taken out, washed thoroughly with water and the surplus water drained off. Fixed by glue on special Specimen, vaporized with an alloy of gold-platinum and dried over night (24 hours) and examined under Scanning Electron Microscope. Particular attention was paid to the structure of external surface, outer and inner chorion layers and the respiratory system between the two layers. In all the eggs the inner layer of chorion is thick and solid but radially traversed by numerous very fine pores. The outer part of the chorion is variously constructed, and it is in this layer that the principal differences occur. Main difference found in these structures show fundamental differences between subfamilies and species. A key for the identification of the egg is provided. This research work was carried out at Department of Zoology, University of Nottingham, England. I am thankful to Dr. J.C. Hartley for providing me research facilities.

**PREVALENCE AND DISTRIBUTION OF HOVERFLIES (DIPTERA: SYRPHIDAE:
ERISTALINAE) OF SUKKUR DIVISION, SINDH, PAKISTAN**

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Hoverflies or flower flies are very important flying insects due to their multidimensional services in ecosystem. They perform the function of pollination and many species act as vital biological control agents against crop pests. Sukkur division is a very rich agro-ecosystem where hoverfly species are widely distributed and are abundant in number. A study was carried out from August 2015 to February 2016 in various localities of the Sukkur Division including Ahmedpur, Pir-jo-Goth, Salehpat, Pano Aakil, Daharki and Mehrabpur. In this survey, five species belonging to sub-family Eristalinae were collected and the total number of specimen was 480. These include *Eristalinus aeneus*, *Eristalinus megacephalus*, *Mesembrius bengalensis*, *Syrirta pipiens* and *Syrirta orientalis* having different host plants. Among these, 55% of specimen belonged to *E.aeneus* and *E.megacephalus*, 15% to *M.bengalensis* and 30% to *S.pipiens* and *S.orientalis*. Whereas, out of 480 specimen, the number of male and female population was 255 and 225 respectively. The results indicate that these species have rich Biodiversity and are first time reported from this region.

**PREVALENCE OF APHIDOPHAGOUS HOVER FLIES OF SUB FAMILY SYRPHINAE ON
DIFFERENT CROPS IN HUB CHOKI, BALUCHISTAN**

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Pakistan is an agriculture country but unfortunately most of the crops are attacked by many insect pests. Aphids are one of them which are polyphagous and attack many food crops. In Hub Choki, Baluchistan mostly wheat, brassica, corn, plums, cheekoo, and date palm trees are grown. Different aphid species. *Lipaphis erysimi*,

Brevicoryne brassicae, *Schizaphis graminum* and *Myzus persicae* were attacking the inflorescences, leaves and stems of plants, results in total discoloration and destruction of that part of plant body and ultimately affect the production of that crop. As recent investigation has confirmed that the larvae of most of the Syrphid flies specially Syrphinae are predator over many phytophagous insects, keeping in view the current investigation is carried out from Hub Choki, a coastal area of Baluchistan, Pakistan, which aimed at finding out the prevalence of different species of aphidophagous hover flies. In current study a total of 1278 specimens of hoverflies belonging to four species, *Episyrphus balteatus* (703), *Eupeodes corollae* (175), *Sphaerophoria scripta* (223) and *Ischiodon scutellaris* (177) were collected from brassica, wheat, corn plums, cheekoo and date palm tree with the help of sweep net, malaise trap and hand picking. Through investigation it was found that the host plants and aphid density had strong positive relation with prevalence of hoverflies. Moreover we also found two new host plants, cheekoo and date palm trees. This paper is part of the higher education commission, islamabad funded project no: 20-3838/NRPU/R&D/HEC/14.

**SCANNING ELECTRON MICROSCOPY OF SHEET WEB OF INDIAN COOPERATIVE SPIDER
(*STEGODYPHUS SARASINORUM*) AND THE EFFECT OF WIND STRESS ON THE
MORPHOLOGICAL FEATURES OF SILK FIBERS**

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Spiders are most diverse group of invertebrates which are inhabited in almost all the terrestrial environments. Almost 46739 species of spiders are distributed worldwide. Sub adult Indian cooperative spiders (*Stegodyphus sarasinorum*) spiders weave sheet web for prey capturing. Spiders were captured and maintained in the laboratory. We studied different structures of sheet web through scanning electron microscopy. Rounded, bilateral, bifurcated, meshwork of tiny fibers, network of extended silk fibers with ribbon clumps zig zag fibers and, thread droplets enwrapped with tiny silk fibers were observed in the webs of these spiders. In the next set of experiments, the effect of wind stress on the morphology of silk fibers was also observed. Spider groups were exposed to variable wind stress and compared with control group. Group II (95% CI: 1.6908, 2.0519) and Group III (95% CI: 1.7140, 2.0751) had significantly higher diameter ($p < 0.05$) as compared to the Control (95% CI: 1.1938, 1.5574) and Group I (95% CI: 1.3939, 1.7550). No significant difference ($p > 0.05$) was observed among Groups II, III and IV, respectively. However, meshwork of tiny fibers was increased in all treatment groups. Thread droplets were very rare in colonial spiders which were only observed in wild silk fibers samples. Small silk droplets were observed in the control group in the laboratory which were absent in the treatment groups. The meshwork of tiny fibers was only present in colonial spiders which covered the axial fibers. The tiny fibers which are the meshwork of puffs in *Stegodyphus sarasinorum* are rarely observed in other spiders. It is predicted that in future we would be able to understand spinning behaviour of ceribellate gland which is the smallest gland in *Stegodyphus sarasinorum* to investigate the mechanical properties of these fibers. The understanding of mechanical properties of these fibers especially small wool like puff may lead to the synthesis of nanoscale fibers in future.

**BIODIVERSITY OF SHORT HORNED GRASSHOPPERS FAMILY ACRIDIDAE
(ORTHOPTRA) FROM DISTRICT DADU SINDH**

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Dadu is located in middle of sindh province of Pakistan. Its geographical location is consisted of river Indus belt from Kati Jatoi, Moro Dadu to Talty to Sehwan, agricultural area in all talukas (Mehar, K.N Shah, Dadu and Johi) cultivation of cereal, vegetables, fruits and cash crops and desert or mountains in western side having wild vegetation on rain water. Many sub family of family acriditae having many genera in Dadu in all fields. Researchers have worked on these genera and species in various research papers. Dadu links with

Balochistan area to the western side kirthar mountain range this area is dry and arid with sand area like desert and hilly area, north side with Larkana, Kamber Shahdadkot districts, southern with district Jamshoro. So Dadu is rich area for grasshoppers.

OLIAGE INSECTS DIVERSITY AMONG OPEN AND RESTRICTED CITRUS ORCHARDS

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The present research conducted to record the "Foliage insects' diversity among open and restricted Orchards" for future planning in restricted and Non-restricted fields. Totally from both fields 1192 specimens were recorded. During current study, from restricted and Non-restricted fields taxa composition is also recorded. Entire population was recorded from Restricted fields, pertaining to 73 species, genera 59, 34 families and 6 orders. Where the population recorded from the Non-restricted fields, pertaining to 71 species, genera 62, 7 orders and 37 families. Species abundance was recorded maximum from restricted and Non-restricted fields, at 40°C temperature and 20% humidity during the 5th sampling (15 species), and during 6th sampling 6 species at 35°C and 28% humidity least abundance was recorded. Maximum population was recorded during the 9th, 6th and 10th sampling (16 Species) in case of Non-restricted fields, at 31°C, 37°C, and 32°C, 76, 29 and 61% humidity, respectively, during 2nd sampling 7 species 91% humidity and 13°C temperature least abundance was recorded. During the 8th sampling (2.08 ± 0.87) at 24°C and 59% humidity in case of Restricted fields maximum biomass recorded, and during 10th sampling (1.03 ± 0.13) at 26°C and 57% humidity least biomass recorded in case of Non-restricted fields, least biomass was recorded during 10th (2.08 ± 0.27) 81% humidity and at 21°C, maximum biomass recorded during 6th sampling (1.53 ± 0.63) 29% humidity and at 37°C temperature. From the total 71 recorded families 71, from restricted fields 34 families were recorded. For family Psyllidae 36.27% (N = 202), relative abundance was recorded extraordinary and in case of non-restricted fields, from recorded families total was 71, from non-restricted fields 37 families were recorded extraordinary for family Psyllidae 44.25% (N = 281) relative abundance was accessed and recorded. Total recorded order was 7, relative abundance was recorded extraordinary for order Hemiptera (Bugs) 43.63% (N = 243) and 6 orders were recorded, then followed by Diptera (True flies) 34.65% (N = 193), Coleoptera (Beetles) 10.77% (N = 60), Hymenoptera (Ants) 7.90% (N = 44), Araneae (Spiders) 2.69% (N = 15). Wherein a Neuroptera (Lacewing) order was not recorded from restricted fields. All the (7) orders were recorded and relative abundance was recorded extraordinary in case of non-restricted fields for order Hemiptera (Bugs) 47.56% (N = 302), followed by Hymenoptera (Ants) 17.17% (N = 109), Diptera (True flies) 15.75% (N = 100), Araneae (Spiders) 10.08% (N = 64), Neuroptera (Lacewing) 3.15% (N = 20). Highest (2.1393) Diversity (H) was recorded from restricted fields as compared to (1.1588) Non-restricted field. In restricted field (20.4177) Richness (R) was recorded maximum and from non-restricted field (19.3857) least. Among both fields (restricted and non-restricted) showed non-significance result (F = 0.04; P = 0.8376) analysis of variance (ANOVA). Taxa composition as well as structural community were differing significantly among both fields F = 12.00; P ≤ 0.0180) Linear Regression confirmed that.

A NEW SPECIES MARVA SINDHENSIS SP.NOV (DERMAPTERA) FROM SINDH PAKISTAN

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The Earwigs belongs to order Dermaptera are of considerable economic importance. It is important to identify then accurately so that diagnosis of economic problem can be properly made the earwigs have been recorded as the pest of Irish sweet potatoes in storage and damaging the roots of vegetables grow in green houses.

SECTION – I V

PARASITOLOGY

NEW SPECIES OF GENUS *SUBULURA* MOLIN, 1960 (NEMATODA: SUBULURIDAE) FROM POULTRY BIRD *GALLUS DOMESTICS* OF DISTRICT KHAIRPUR, SINDH, PAKISTAN.**Hafeeza Gul^{1*}, Nadir Ali Birmani², Abdul Manan Shaikh¹, Saeeda Anjum Buriro² and Shabana Mangi¹.**¹Department of Zoology, Shah Abdul Latif University, Khairpur²Department of Zoology, University of Sindh, Jamshoro

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During current investigation on helminth parasites of Domestic Fowl 50 hosts were randomly collected from different localities of district Khairpur. Alimentary canal, liver, gallbladder, lungs, kidneys and body cavity were examined under stereodisecting microscope for the presence of nematode parasites. Amongst these hosts examined 300 specimens (70 ♂ and 230 ♀) of nematodes belonging to genus *Subulura* Molin, 1960 recovered from intestine and gizzard of 60 hosts. Present specimens come closer to all the known species of genus *subulura* but differ in arrangement of precloacal papillae, post cloacal papillae and cloacal papillae, shape of gubernaculum; position of valvular opening; and varying size of diagnostic characters other uniqueness. Hence the specimens identified as new species *S. aligulabi* sp. The name of new species *S. aligulabi* refers the name of father of the first author.

FIRST RECORD OF *ASTIOTREMA TIGRINUM* (TREMATODES ASTIOTREMATINAE) IN PAKISTAN FROM *HOPLOBATRACUS TIGERINUS* (AMPHIBIANS: DICROGLOSSIDAE)**Attia Bushra*, Nadir Ali Birmani and Ali Murtaza Darejo**

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A survey was conducted to study helminth parasites of frogs (*Hoplobatrachus tigerinus*) from district Umerkot Sindh Pakistan. A total of eighteen hosts *Hoplobatrachus tigerinus* were collected from different aquatic habitats for the presence of helminth parasites. During examination of visceral organs and gut seven trematodes be a member of Genus *Astiotrema* Loss, 1900 were recovered from the intestine and identified as *Astiotrema tigrinum* n.sp. On the basis of body shape; terminal oral sucker; laterally elongated, overlapped pharynx; absence of prepharynx; highly muscular hook-shaped cirrus sac; ventral sucker separated from ovary with cirrus sac; lobed testes; extension of vitellaria from posterior margin of ventral sucker reaching up to anterior testis; ceca extended up to anterior testis and shorter post-testicular space, a new species *Astiotrema tigrinum* is proposed. The name of new species refers to the species name of the host.

FIRST RECORD OF GENUS *COSMOCEPHALUS* MOLIN, 1858, IN *PASSER PYRRHONOTUS* BLYTH, 1845 (PASSERIDAE: PASSERIFORMES) FROM SUKKUR, SINDH, PAKISTAN.**Irshad Chandio*, Ali Murtaza Dharejo, Muhammad Munif Khan and Saima Naz**

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During current studies on the helminth parasites of nematodes of *Passer pyrrhonotus* BLYTH, 1845, total ten nematodes (three ♂ and seven ♀) were collected from the intestine of the four host, killed and preserved in 70% Ethanol and Glycerol solution for detailed study. Measurements are taken in millimeters (mm) and eggs in micrometers (µm). The genus *Cosmocephalus* Molin, 1858 and *Passer pyrrhonotus* is a new host reported for the first time from Pakistan.

CAMEL MANGE: A COMMON SKIN PROBLEM IN THARPARKAR ECOSPHERE**A.G. Arijo, B. Narejo*, N. Akhter, Shahjahan K.K and Shayan Ahmad Arijo***Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam-Pakistan-70060*

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Random skin examination of 150 camel out of a total of 2500 camel of both sex and all age groups was carried out in Mithi, Islam Kot, and Nangar parker regions of Tharparkar and 100 percent examined camel were found to have infestation with mites. Skin scraping processed for laboratory diagnosis of mite species revealed presence of only one species i.e. *Sarcoptes scabiei* in all camel during study. Rate of infestation was higher in juvenile and females when compared with males and intensity of sever skin condition was age associated. Face, neck abdomen forelimbs and hind limbs were found to have clear symptoms of scabies. Hind legs had worst condition as compared to other parts of the body. Crude Diesel, neem leaves are used as local wisdom for the treatment of ailment, but intensity of infestation remains same since years. High humidity, rainfall and low temperature are stimulating abiotic factors contribution towards increasing the severity of infestation. Herders claimed of increased irritation, itching induced in all family members during housing of infested camel near houses, whereas, these medical conditions were reported to be absent while camel were out of grazing on xerophytes. There is dire need of veterinary services to control the problem that has gone to the point of no return.

A NEW SPECIES OF GENUS *RHABDOCHONA* RAILLIET, 1916 (NEMATODA: RHABDOCHONIDAE) FROM SINDH, PAKISTAN**Hira Soofi*, Nadir Ali Birmani, Ali Murtaza Dharejo and Abdul Rasool Abbasi***Department of Zoology, University of Sindh, Jamshoro*

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During parasitological study on Siluriformes catfish host *Rita rita* were collected from Indus river Jamshoro Sindh, Pakistan. A total of 125 nematodes (70 ♀ & 55 ♂) were collected from intestine of hosts. Live nematodes were killed in hot 70% ethanol, cleared in lactophenol and glycerol solutions and preserved in alcohol-glycerol solution for further examination. Present nematodes were identified as new species *Rhabdochona indusi* on the basis of morphometric differences such as large striated body, greatest width in posterior region of male and anterior half of the female, 19 pairs of papillae, spicule size and shape, large spicule rounded anteriorly with small groove at lateral side and pointed posteriorly with curved ventrally without uniform thickness, smaller spicule rounded anteriorly and pointed posteriorly, shape of genital structures vulva marginal; anterior vulvar lip hook shape and posterior vulvar lip pointed, vagina muscular directed posteriorly, shape and size of eggs. The name of new species refers to the Indus river from where the host fishes were collected.

A NEW SPECIES OF GENUS *THAPAROTREMA* GUPTA, 1955 (TREMATODA: OPISTHORCHIIDAE) FROM CATFISH *RITA RITA* (SILURIFORMES: BAGRIDAE) OF SINDH, PAKISTAN**Hira Soofi*, Nadir Ali Birmani, Ali Murtaza Dharejo and Abdul Rasool Abbasi***Department of Zoology, University of Sindh, Jamshoro.*

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The catfishes *Rita rita* were collected from Indus river Jamshoro, Sindh, Pakistan as a host for examination of helminth parasites during current research study. A total 5 hosts were infected with 7 trematodes belonging to genus *Thaparotrema* from 23 hosts. Trematodes were compare with its closest allies using literature and identified as new species *Thaparotrema shamimi*. The new form differs from its congeners in having body shape and size, posteriorly narrow and strongly curved at level of ovary, oral sucker terminal and

rounded with curved at posterior level, ventral sucker oval in shape, anterior testis square in shape and posterior testis triangle in shape, ovary size and position, seminal receptacle oval in shape, uterine coils highly condense, seminal vesicle shape and position. The name of new species refers to the honored of author's mother name Soofi Shamim.

**EFFECT OF SOME BIOFERTILIZERS, PLANT NUTRIENTS AND A BIOCIDES FOR
MANAGEMENT OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* INFECTING
SUNFLOWER**

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The reniform nematode, *Rotylenchulus reniformis* attacks a wide range of crops including sunflower, *Helianthus annuus* in Egypt as well as in many parts of the world. Elimination of the nematodes has received attention to minimize damage to plants. Thus, the present study reports the probable effects of some biofertilizers, plant nutrients and a biocide on the development of *R. reniformis* in sunflower and growth of the plant. Three Egyptian bio-fertilizers (BF), i.e. Nitroben, Rizobactrein and Blue-green; and three Egyptian plant nutrients (PN) i.e. Citreine, Kotangein and Kapronite as well as the biocide Nemaless were evaluated at three rates (a lower rate, the recommend rate and a higher rate) for control of *R. reniformis* and improvement of sunflower cv. Giza 101 under greenhouse conditions 30 ± 5 °C. All the evaluated compounds significantly reduced ($P \leq 0.05$ and/ or 0.01) the number of juveniles in soil, swollen females and egg-laying females on roots. The reduction varied greatly according to the type of experimented products and rate of application. The highest reduction in the nematode populations, swollen females and egg-laying females was attained with seed coating by Rizobactrein followed by Nitroben as bio-fertilizers while, the least reductions were obtained by using Nemaless as a biocide followed by Blue-green as alga biofertilizer. Application of the plant nutrients, Kapronite as soil amendment and Kotangein as seed coating effectively decreased the development of the nematode stages. Citreine as a foliar spray nutrient was the least effective. Generally, Rizobactrein and Nitroben as biofertilizers; Kapronite and Kotangein as plant nutrients proved to be the most effective for controlling *R. reniformis* and gave the greatest growth of sunflower plants as compared with the rest treatments.

**APATEMON SP. SZIDAT, 1928 (TREMATODA: STRIGEIDAE) FROM THE BIRD
BUBULCUS IBIS (CATTLE EGRET) IN NAUSHARO FEROZE, SINDH, PAKISTAN**

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In result of ongoing helminthological studies on two *Bubulcus ibis* (Cattle egret) were shot down from the District Nausharo Feroze, Sindh, Pakistan and brought to the Parasitology laboratory, Department of Zoology, University of Sindh, Jamshoro. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. Eight specimens were collected from the two small intestine of *Bubulcus ibis* and mounted permanently according to standard procedure. A detail study was conducted and identified as belonging to genus *Apatemon* sp. The genus is characterized by having: Body bipartite, small. Fore body is infundibular, hind body is sub-cylindrical, less arched, pharynx well developed, the intestinal caeca are long and proceed up to in-front of posterior end of hind body, the tribocytic organ is multi-lobed and occupies greater part of the fore body, oral sucker oval in shape, well developed, terminal, wider than long, ventral sucker roughly tri-angular in shape, testes tandem, inter-caecal, situated in posterior half of hind body and overlapped by each other, anterior testis appears to be slightly with rough indentations and smaller in size than posterior testis, seminal vesicle situated below the posterior testis, copulatory bursa has an inverted cup-shape in the posterior most part of the hind

body. ovary pre-testicular, oval to rounded, follicular vitellaria is confined to the ventral and sub-ventral fields in the hind body, extending from near the anterior end of hind body up to a little in front of its posterior end. Eggs are oval, double walled. The specimens studied are therefore not designated up to species level and slight differences recorded. Present specimens are recovered in *Bubulcus ibis* (Cattle egret) a new locality i.e. Nausharo Feroze Sindh, Pakistan.

**STEPHANOPRORA LARKANENSIS SP.N. (TREMATODA: ECHINOSTOMATIDAE) FROM THE
INTESTINE OF VANELLUS INDICUS (REDWATTLED LAPWING) IN LARKANA, SINDH,
PAKISTAN**

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Three birds *Vanellus indicus*, Redwattled lapwing were caught alive from District Larkana, Sindh, Pakistan and brought to the Parasitology laboratory, Department of Zoology, University of Sindh, Jamshoro, Pakistan. The morphometric of birds were recorded and then anesthetized, dissected and examined for collection of internal helminth parasites. All birds were infected with ten specimens. The worm was mounted permanently according to standard procedure for further detail study and identified as belonging to genus *Stephanoprora* Odhner, 1902 and proposed as *Stephanoprora larkanensis* sp.n. The new species is characterized by having: Body elongate, anterior and posterior ends are narrower while middle of the body is broader, maximum width is attained at the acetabular level, head collar small, reniform, have 26 spines. Oral sucker terminal, well developed, oval to rounded in shape, pharynx muscular, oval, elongate, esophagus moderately long, bifurcates near mid of the ventral sucker into two intestinal caeca, acetabulum much larger than the oral sucker, rounded, highly muscular situated in the anterior part of the body, Ovary median, pretesticular, without lobes, oval to rounded, little in-front of the anterior testis, testes post-ovarian, median, tandem situated in the middle of the body, cirrus pouch plump shaped, anterior to the acetabulum, vitellaria extend in the lateral fields starting from below the acetabulum up to the posterior part of the body, uterus short, with loops, between the ovary and acetabulum, eggs oval, thin walled. The species name refers to the host locality.

THREATENING CLOUDS OF LEISHMANIASIS IN MULTAN

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Leishmaniasis, a parasitic disease of humans serving to be a challenge to control as its geographic distribution is expanding to considerable extent in Pakistan and worldwide. The disease is vectored by phlebotomine insect, commonly known as Sand Fly. Causal organism of the disease is a protozoon called *Leishmania* spp. transmitted in people during sucking of blood by the pest. Various forms of the disease are found in the world but the most common are Cutaneous Leishmaniasis (CL), Visceral Leishmaniasis (VL) and Muco-Cutaneous Leishmaniasis (MCL). The present study was conducted to access the number of reported patients in two public sector hospitals of Multan in 2016. A substantial number of patients were reported in Out Patients Departments (OPDs) of the hospitals. Among the reported cases, 390 patients showed the symptoms of CL, while no other form was found. According the data, infection was found a slightly higher in females (51.0 %) than in males (49 %). While considering the age, full-grown adults (35 years) were affected more, whereas in teen-agers with the age of 18 were infected more. Most of the lesions were found on hands and arms of the patients and a maximum of 9 lesions were observed on the body of a single patient. It can be concluded that the vector of Leishmaniasis is present in Multan and this is the time to educate the public about the disease and its prevention.

A REVIEW OF HELMINTH PARASITES OF FRESH WATER FISHES OF INDUS RIVER, PAKISTAN

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River Indus is an important river of Pakistan abounds with extensive biodiversity. Its fauna especially fish fauna have nutritional and commercial value. However, Helminth parasites badly affect fish health and decrease its nutritional and commercial importance. In order to assess the existing research about presence of helminths parasite of fish water of Indus River, a comprehensive review of research papers, bulletin and thesis conducted. It is found that there is dearth of parasites research of fresh water of Indus River. Moreover, present review will help research to plan project and develop link with government department for immediate measure to address the problem.

HAEMATOLOGICAL CHANGES IN FRESHWATER FISH *CHANNA PUNCTATUS* (BALOCH 1973) INFECTED WITH ACANTHOCEPHALAN PARASITES

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The freshwater fishes are an important source of animal proteins to human. They are adversely affected by many helminth parasites. Present study was conducted to determine the changes in haematological parameters of the fresh water fish *Channa punctatus* (Bloch 1973) naturally infected with acanthocephalan parasites. For this, 140 *channa punctatus* fishes with average body weight of 257±8.8 g were examined. The haematological indices were assessed and significant changes in the haemoglobin contents, total erythrocytes and leukocyte count, mean corpuscular volume, mean corpuscular haemoglobin and differential leukocyte count were observed in the infected fishes as compared to non-infected fishes. The results of present study reveals that the parasitic infections adversely affects the haematology of the fresh water fish *Channa punctatus* that may often cause anaemia.

HISTOPATHOLOGICAL CHANGES IN THE LIVER INDUCED BY NEMATODE PARASITES IN CATFISH, *ARIUS THALASSINUS* (RÜPPELL, 1837) FROM KARACHI COAST.

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In the present study the histopathological changes in the liver of catfish, *Arius thalassinus* (Rüppell, 1837) from Karachi coast due to the nematode parasites is described. For sampling purpose, fish were purchased from Karachi fish harbor and brought to the parasitology laboratory for further detailed investigation during February, 2016. Histological sections were prepared by using standard procedures of parasitology. Selected liver tissues were fixed and stained by using eosin and haematoxylin. Six *Raphidascaris acus* (Bloch, 1779) larvae were found attached to visceral mesenteries and liver of *Arius thalassinus* (Rüppell, 1837). The

histopathological changes include loss of hepatocytes architectural morphology, necrotic abnormalities, inflammatory response of the host tissues, blockage of central veins, thickening of arterioles, enlarged cells, disintegration and degeneration of liver cells and tissues were observed.

SUBULURA MOLIN, 1860 (NEMATODA: SUBULURIDAE) REPORT FROM THE GALLUS GALLUS DOMESTICUS (DOMESTIC CHICKEN) IN NAUSHARO FEROZE, SINDH, PAKISTAN

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During survey of Parasitic infection in *Gallus gallus domesticus* (Domestic Chicken) were purchased from the District Nausharo Feroze, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. The intestine of nine chickens were found infected with 201 (45 males, 156 female) specimens. These were fixed in hot steaming 70 % ethanol. Later the nematodes were stored in a solution of 5 parts glycerine and 95 parts 70 % ethanol and cleared in either glycerine or lacto phenol for detailed study. The specimens is characterized by having: These are moderately long, whitish nematodes, tapering posteriorly to a pointed end, while the head region is quite broader and roughly rounded in appearance, lateral cephalic and cervical alae was observed in some male specimens, mouth dorsoventrally elongated with membranous lips, vestibule with chitinous lining, muscular esophagus, dilated posteriorly and followed by a bulb. Male: The spicules are long, stout, alate and equal in size, pre-cloacal sucker fusiform with well-developed chitinous rim, situated some distance above the cloaca provided with a pair of pedunculated papillae each on either side of the sucker. In some specimens, there is pair of pedunculated papillae in the mid of caudal sucker and pair of sessile papillae just behind the sucker, caudal alae absent. Caudal papillae sessile, three pairs pre-anal and six pairs post anal, arranged in two longitudinal rows. Tail is sharply pointed. **Female:** Larger than male, vulva opens near middle of the body from anterior end, the tail is straight, ends in sharp pointed end, eggs are smaller, rounded, sub-globular and thin walled.

COMPARATIVE ANALYSIS OF THE PREVALENCE OF HUMAN PARASITES FOUND IN FRESH VEGETABLES SOLD IN DISTRICT DIR (LOWER) AND DISTRICT PESHAWAR, KHYBER PAKHTUNKHWA, PAKISTAN

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Consumption of raw vegetables in one way is a leading source of transmission of intestinal parasites. This study aimed at determining the prevalence and predictors of parasitic contamination of vegetables collected from two main vegetable markets one each in districts Lower Dir and Peshawar, Northeast of Pakistan. A cross-sectional study was conducted from April 1st to October 30th 2017 to determine the level of parasitic contamination of vegetables sold in districts Lower Dir and Peshawar. A total of 800 samples of different types of vegetables were soaked in physiological saline, followed by vigorous shaking with the aid of a mechanical shaker for 20 minutes and then examined using the sedimentation concentration technique. Out of the 800 samples examined, 19.7% (n=158/800) were contaminated with at least one type of parasite. *Ascaris lumbricoides* 12.3% (n=99/800) was the most frequently detected parasite and *Taenia saginata* 1.62% (n=13/800) was the least frequently detected one. Interestingly, the p value was significant (p>0.05 at 95%CI) between the number of examined and that of contaminated for all the variables studied as education status of the vendors, markets location, type of vegetables, means of display, washed before display, source of water for

washing and market type. The findings of this study provide evidence that there is a potentially high risk of acquiring parasitic infections from the consumption of raw vegetables in Lower Dir and Peshawar districts, Pakistan. The authors believe that an effort should be made by the relevant bodies to reduce the rate of contamination of products with medically important parasites by educating the vendors and the community.

PARASITIC CONTAMINATION OF RAW VEGETABLES COLLECTED FROM SELECTED LOCAL MARKET IN DISTRICT MALAKAND, KHYBER PAKHTUNKHWA, PAKISTAN

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Vegetables eaten raw is the only principal source of transmission of human intestinal parasites. This study was aimed to assess the prevalence and existence of parasitic contamination of vegetables collected from the main vegetable market in district Malakand. A cross-sectional study was conducted from April 1st to October 30th 2017 to determine the level of parasitic contamination of vegetables sold in district Malakand. A total of 630 samples of different types of vegetables were soaked in physiological saline, followed by vigorous shaking with the aid of a mechanical shaker for 20 minutes and then examined using the sedimentation concentration technique. Out of the 630 samples examined, 24.7% (n=156/630) were contaminated with at least one type of parasite. *Ascaris lumbricoides* 16.5% (n=104/630) was the most frequently detected parasite and *Trichuris trichura* 1.42% (n=9/630) was the least frequently detected one. There was significant association between the education level of vendors and the parasitic contamination rate of the vegetables they were selling ($P = 0.0201$). The association between vegetable collected and that of infected was non-significant ($P=0.99114$). Compared to type of vegetable collected mint was highly infected 42.8% while cucumber showed least 11.4% of the parasitic contamination. Regarding the factor means of display of the vegetables ($P=0.0109$), wash before display ($p=0.0026$). The findings of this study provide evidence that there is a potentially high risk of acquiring parasitic infections from the consumption of raw vegetables in district Malakand, Pakistan. The authors believe that an effort should be made by the relevant bodies to reduce the rate of contamination of products with medically important parasites by educating the vendors and the community.

STUDY OF ENDO-PARASITES FROM FAECAL SAMPLES OF BLACKBUCK (*ANTILOPE CERVICAPRA*) KEPT AT JALLO WILDLIFE PARK, LAHORE

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A study was conducted to observe the prevalence of parasitic infection in native Blackbuck (*Antelope cervicapra*), kept at Jallo Wildlife Park, Lahore, to prevent our live stock from being extinct and to save the economic loss of our country. The occurrence and intensity of endo-parasites was determined by standard qualitative and quantitative parasitological techniques. Samples of faecal matter were collected and preserved using traditional method. Faecal eggs count of each sample was determined by Modified McMaster Technique. At the termination of experiment, the data so collected showed significant number of endoparasitic species belonging to different phyla in almost all experimental animals in selected location. From the protozoans prevalence (%) of *Eimeria* spp. is dominant with 81.1% and from helminthes *C. cotylophorum* and *F. magna* with 45.5% prevalence was higher in Blackbuck kept at Jallo Wildlife Park. Overall, two species of Phylum Protozoa (*Eimeria* spp and *Isoospora* spp), 7 species of Phylum Nematoda (*B. trigonocephalum*, *C. ovina*, *G. pachyscelis*, *H. contortus*, *M. marshalli*, *N. spathiger*, *O. circumcincta*), 5 species of phylum platyhelminthes (*C. cotylophorum*, *F. gigantic*, *F. hepatica*, *F. magna* and *P. cervi*) were identified respectively in all samples of Jallo Park. Conclusively, high prevalence of endo-parasites was determined among all animals from the present study indicating life threats and economical loss of natural number of live stock.

HISTOLOGY OF STOMACH OF FISH (*PLECTORHINCHUS LACEPEDE*) SPECIES INFECTED WITH TREMATODE (*PROCTOECES MACULATUS* LOOSS, 1901)

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The fish *Plectorhinchus* Lacepede, 1801 found in fresh, brackish and salt waters is infected with a number of helminth parasites causing tissue damage to various organs. In the present study histological changes caused by *Proctoeces maculatus* (Looss, 1901) to the stomach tissue of the fish is being reported in detail. The fish were caught from Karachi coast, Pakistan. The infected tissue from the stomach was passed through graded ethanol, cleared in xylene, impregnated and embedded in Paraffin wax by using a microtome. Tissues were stained with Haematoxylin and eosin for light microscopy examination. The sections showed atrophic gastritis in the mucosa and several empty spaces were seen in the underlying layers, similarly the architecture of villi was completely destroyed. The present study indicates that tissue was impaired of the host fish.

NEW RECORD OF *ARTYFECHINOSTOMUM INDICUM* (BHALERAO, 1931) MENDHEIM, 1943 (TREMATODA: ECHINOSTOMATIDAE) RECOVERED FROM *MUS MUSCULUS*- HOUSE MOUSE OF DISTRICT NAUSHAHRO-FEROZE, SINDH, PAKISTAN

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The order Rodentia is the most widely existing group on Earth and largest group of small mammals, consist of one forth the total mammalian species of Pakistan. *Mus musculus* (house mouse) is commensal with the human, are omnivore and scavenger in nature. It is the definitive as well as intermediate host for helminths parasites. During the current study, a total number of 32 mice were dissected for the presence of helminthic infection, resulted in 62.5% of helminth infection with 12.5% prevalence of the trematodes. In present work, the most prevalent trematode species recovered was *Artyfechinostomum indicum* (Bhalerao, 1931) Mendheim, 1943 (family Echinostomatidae) and reported for the first time from *Mus musculus* (house mouse) and Sindh province. Previously this species was reported in the duodenum of *Uromastix hardwickii*, India. There is no record of this zoonotic species in Pakistan, therefore, this makes it as new locality and host on record.

NEW HOST RECORD OF *CATATROPIS PAKISTANENSIS* (CREPLIN, 1925) (TREMATODA: NOTOCOTYLIDAE) FROM FERRUGINOUS DUCK *AYTHYA NYROCA* GULDENSTADT (ANSERIFORMES: ANATIDAE) IN SINDH, PAKISTAN

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During helminthic studies on the migratory birds in Sindh, Pakistan, total of six ferruginous duck *Aythya nyroca* (Guldenstadt) were captured from the Hamal Lake, district Shahdad Kot in December 16 to February 17. During examination of visceral organs, 10 trematodes of *Catatropis pakistanensis* (Creplin, 1925) were recovered from intestine. All trematodes were processed according to the procedures given by Garcia and Ash, 1979 and mounted permanently in Canada balsam. These trematodes were recovered from pochards for the first time in the region, making new host as well as new locality record for this species of trematode.

EPIDEMIOLOGICAL STUDIES OF GASTROINTESTINAL NEMATODES IN SMALL RUMINANTS OF DISTRICT POONCH, AZAD JAMMU AND KASHMIR

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Gastrointestinal nematodes are internal parasites also known as roundworms. Gastrointestinal nematodes are considered to be most economically important endoparasites of sheep and goats reported to be responsible for causing infection in goats and sheep globally. There is no published data available on the epidemiology of gastrointestinal nematodes in district Poonch, Azad Jammu and Kashmir. Therefore recent study was designed to work out the epidemiology of gastrointestinal nematodes and identification of gastrointestinal nematodes of small ruminants in district Poonch. Study was conducted from 2015 to 2016 in different regions of district Poonch. For this small ruminants (goats and sheep) were selected randomly from the study area. Abomasums were collected from 724 goats and sheep for the observation of gastrointestinal parasites. Direct observation of abomasal parasites were done following standard protocol. Results showed overall prevalence of gastrointestinal parasites was 81% (588/724) in which 87% (397/459) in goats and 72% (191/265) in sheep on the basis of abomasal analysis. Relatively high prevalence of 87% (397/459) nematodes was found in goats than sheep 72% (191/265). Study revealed that high prevalence was found in female goats than male goat. Similarly female sheep suffered more than male sheep. *Haemonchus contortus* was the most prevalent parasite encountered in different regions of district Poonch followed by *Ostertagia* and *Trichostrongylus*. Mixed infection of these small parasites was also found. High prevalence was found in spring season. Goats had large dominance of *H. contortus* than sheep. Region wise result showed high predominance of gastrointestinal parasites in Rawalakot region followed by Hajira, Abbaspur and Paniola region. Least prevalence of gastrointestinal parasites was observed in Thorar region in both male and female sheep and goats.

DETECTION OF MULTIPLE ANTHELMINTIC RESISTANCE IN GOATS DISTRICT PALLANDRI, AZAD JAMMU KASHMIR

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Goats are vital part of livestock and agriculture sector which help in national economic development throughout the world. Goats are managed for leather, milk, and meat purposes. The possible production of goats is largely affected by the presence of multiple anthelmintic resistances in them. The current study has been planned to detect multiple anthelmintic resistance in gastrointestinal nematodes of goats managed at various parts of Pallandri Azad Jammu and Kashmir. *In vivo* evolution of multiple anthelmintic resistance were done by using Faecal Egg Count Reduction Test, Mean faecal egg count, percentage reduction and 95 percent confidence level was calculated by the help of the formula suggested by the World Association for the Advancement of Veterinary Parasitology for evaluating multiple anthelmintic resistance. Two goat herds were classified into four different groups for treatments and each group contain 10 goats: Albendazole, Levamisole, Mixture of Albendazole and Levamisole and a control group treated with water. The percentage decrease in fecal egg count (95 percent confidence intervals) for Levamisole, Albendazole and mixture of both flocks I were found 91.0 %, 91.0 % and 95.0 % and for Flock II were 90.0 %, 91.0 % and 95.0 % (Efficacy in percent). Results of Faecal Egg Count Reduction Test show a significant difference ($P < 0.05$) on pre treatment and post treatment with treatment groups as contrary to control group of treated with water. The results revealed that the

presence of multiple anthelmintic resistance in experimental flocks. Two herds were detected for resistance compared to the Albendazole; two were found resistant against the levamisole, sensitive for resistance against mixture of both Albendazole and Levamisole. Control efficacy test results revealed that presence of gastrointestinal nematodes of species *Haemonchus contortus*, *Ostertagia circumcincta* and *Strongyloides* species.

A NEW SPECIES OF GENUS *BRUEELIA* KELER, 1936 (PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) ON COMMON MYNA, *ACRIDOTHERES TRISTIS* (PASSERIFORMES: STURNIDAE) FROM SINDH, PAKISTAN

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The genus *Brueelia* Keler, 1936 occurs primarily in songbirds and is one of the most specious group of chewing lice, with over 300 described species. The Common Mynah, *Acridotheres tristis* (Linnaeus) (Passeriformes: Sturnidae) was previously reported to three species. During present study, 12 mynas, *Acridotheres tristis* were examined from Hyderabad, Sindh, Pakistan, in which the bird host was reported to be infested by four species of genus *Brueelia*. Presently, one species of the genus *Brueelia* was studied and identified with the help of its specific characters. Collected specimens of genus *Brueelia* were mounted permanently in canada balsam and studied thoroughly under microscope for their identification. The present new species differs from its congeners in having shape of dorsal anterior head plate, abdominal tergites and chaetotaxy, subgenital plate of male and female, terminal chaetotaxy of female and male genital structure. On the basis of these characters the new species has been proposed as *Brueelia gustafssoni*. The name of the new species has been given in the honour of a well-known chewing lice specialist, Prof. Daniel R. Gustafsson who helped during identification and determination of the *Brueelia* species of Common Myna.

COPROLOGICAL STUDY ON PREVALENCE OF GASTROINTESTINAL PARASITES IN CATTLE IN DISTRICT SIALKOT

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This Study was conducted to determine the prevalence of parasitic infestation in cow, buffalo, sheep and goat population of Sialkot district. From September 2016 to august 2017 total 4819 fecal samples were examined by floatation and sedimentation method at animal disease diagnostic lab Sialkot. 4509 (93.56%) samples were positive for parasitic infection. Total number of samples examined from cows, buffalos, sheep and goat were respectively 1639 (34.01), 3036 (63.00%), 61(1.26%), 83 (1.72%). out of 1639 cow samples 1534 (93.59%) were positive for parasitic infection. 1420 (92.56%) milk producing cows, 62 (4.04%) cow heifer, 48 (3.12%) cow calves were positive. Parasitic load was found to be 36.64%, 35.41%, 8.56%, 5.03%, 3.12%, 11.21% for fasciola, nematodes, cocci, par amphistomum, cestodes and any others respectively. This heavy load of worms causes poor health conditions of cattle leading to decreased and low quality meat and milk production that ends up with great economic loss to the keepers. Consumption of unhealthy meat and milk also poses serious health risk to the public. Public awareness programmes about safe husbandry and better housing techniques may prove beneficial.

**A NEMATODE RECOVERED FROM COMMON TEAL, *ANAS CRECCA* L.
(ANSERIFORMES: ANATIDAE) FROM SINDH, PAKISTAN**

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In present study on nematological studies of common teal *Anas crecca* L. (Anseriformes: Anatidae), a total of 40 hosts were collected from Manchar Lake of District Dadu and were examined by autopsy for the presence of helminthic infection. The infection of nematodes was observed maximum (80%) among all helminthes in the host species. Furthermore, there is no significant work has been done on nematodes from this host in Pakistan, which revealed the first record of a nematode, *Amidostomum acutum* (Lundahl, 1848) Seurat, 1918 from *Anas crecca* in Sindh region, making new host and new locality record.

**INCIDENCE OF PARASITIC AND FUNGAL INFECTION IN SILVER SHARK,
*BALANTIOCHEILUS MELANOPTERUS***

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An aquarium is a type of Ex-situ conservation which provides not only amusement but also information on behaviour and biology of ornamental fishes. The aim of present study was to investigate the parasitic and fungal infection of imported Silver shark, *Balantiocheilos melanopterus*. Parasites were isolated from different organs of fish samples and identified under microscope. While fungi were isolated, grown in SDA media followed by pure culturing technique reveals their identification under microscope with aid of genus-specific colony morphology. The standard protocol identified a monogenean *Dactylogyrus* sp. for parasitic infection in 12 out of 20 specimens with prevalence rate, abundance and mean intensity of 60%, 18.5 and 30.83. The highest parasitic load was found in Body weight class (2.0-3.0 g) 155 and in Body length class (6.1- 6.5 cm) which was 227. The most abundant fungal genera was *Aspergillus* followed by *Alternaria*, *Mucor*, *Rhizopus* and *Penicillium*. Monogenean causes degeneration and hemorrhages on gill filaments and are host specific as our study also showed highest proportion of *Dactylogyrus* sp. was located on the second gill arch which justifies its microhabitat specificity. Poor aquarium management aided by lack of basic health management practices along with uncontrolled import of live fish into the country can lead to transmission of pathogenic parasites to native fishes, causing a great economic and ecological threat to valuable native fishes. It is strongly suggested to assess the threat well in advance, in order to protect native species and the ecosystem.

**PREVALENCE OF HELMINTH PARASITES IN FRESHWATER FISHES OF RIVER INDUS
COLLECTED AT JAMSHORO, SINDH, PAKISTAN**

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In result of ongoing research project on the Biodiversity of helminth parasites of freshwater fishes of River Indus in Sindh province, Pakistan, a total of 43 fishes were examined. Only four species of freshwater fishes including *Wallago attu*, *Lamnostoma kampeni*, *Mystus cavasius* and *Cirrhinus reba* were captured live from the river Indus at Jamshoro and examined for the presence of helminth parasites. These fishes were examined under stereodissecting microscope which revealed highest prevalence for nematodes (27.43%) followed by trematodes (25.66%). In trematodes, the highest infestation was recorded in *Wallago attu* that was (100%), followed by *Lamnostoma kampeni* that was (55.55%) and minimum infestation was recorded in *Mystus cavasius* that was (54.54%), whereas, *Cirrhinus reba* was found negative. In trematodes, the highest infestation

was recorded in *Mystus cavasius* that was (45.45%), followed by *Lamnostoma kampeni* that was (9.09%), whereas, *Wallago attu* and *Cirrhinus reba* were found negative.

A NEW SPECIES OF GENUS *LAMPROGLENA* (COPEPODA: CYCLOPOIDA: LERNAEIDAE) FROM SNAKEHEAD FISH (*CHANNA STRIATA* BLOCH, 1793) FROM SINDH, PAKISTAN

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One new species of copepods has been recovered from the gill-filaments of snakehead (*Channa striata* Bloch, 1793) fish in pond of Khipro, District Sanghar, Sindh, Pakistan. 28 fishes were examined for their ectoparasites, in which 07 fishes were found infested with 26 specimens of copepods, genus *Lamproglena*, and belonging to family Lernaecidae. The present new species has been identified by taxonomic keys, literature review, diagrams and photographs and was compared with its related species, on basis of which it was identified as new. The name of new species was proposed on the locality province from where it has been collected and described as *Lamproglena sindhensis* spcies novum.

PREVALENCE OF ACANTHOCEPHALAN IN FRESHWATER FISH *CATLA CATLA* (HAMILTON, 1822) OF RIVER INDUS AT JAMSHORO, SINDH, PAKISTAN

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In result of ongoing research project on the Biodiversity of helminth parasites of freshwater fishes of River Indus in Sindh province, Pakistan, a total of five freshwater fishes *Catla catla* (Hamilton, 1822) of river Indus at Jamshoro were collected and examined for the presence of acanthocephala. The examination of gut contents and visceral organs of *Catla catla* revealed three fishes were harboring the acanthocephalan parasites. All specimens of acanthocephala were collected from the intestine of the host fishes. Previously there is no record of acanthocephala of *Catla catla* of river Indus at Jamshoro.

***TETROCHETUS BALOCHISTANENSIS* N.SP. (DIGenea: ACCACOELIIDAE) FROM DOLPHINFISH *CORYPHAENA HIPPURUS* (PERCIFORMES: CORYPHAENIDAE) OF GWADAR COAST, BALOCHISTAN, PAKISTAN**

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Coryphaena is a genus of marine ray-finned fishes known as the dolphinfishes. Currently it is only known genus in the family Coryphaenidae. Dolphinfishes are some of the fastest-growing species in the ocean, serve as a primary food source for many pelagic predators and can reach up to about 88 pounds weight. There is no record on the helminth parasites of marine fishes especially trematode parasites in Balochistan with special reference to Gwadar. Present study identifies a new species of trematodes collected from the host fish on the basis of muscular, robust and smaller body; oral sucker smaller, subterminal; testes oval-shaped, preovarian in position, situated on lateral sides, disposed in mid-point of body; ventral sucker almost equal to oral sucker; ovary oval-shaped, median, close to posterior testis and vitellaria densely distributed, tubular in shape, extending from mid-level of ventral sucker reaching up to anterior margin of ovary. The name of new species refers to the Baluchistan province from where the host was collected.

**PREVALENCE OF NEMATODE PARASITES IN SPINY EEL *MASTACEMBELUS ARMATUS*
(SYNBRANCHIFORMES: MASTACEMBELIDAE) OF RIVER INDUS AT
THATTA DISTRICT, SINDH, PAKISTAN**

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Present study is based upon the prevalence of nematode parasites in Spiny eel, *Mastacembelus armatus* (Lacepède, 1800) collected from different localities of River Indus at Thatta district, Sindh, Pakistan. During August to December, 2017, a total of 15 fishes were examined for the presence of nematode parasites. The overall prevalence was (66.66%). These nematodes were collected from intestine, gills and liver. Previously, there is no record on the nematode parasites of Spiny eel, *Mastacembelus armatus* from this study area.

***CONSPICUUM ALIRAAZI* N.SP. (TREMATODE: DICROCEOLLIDAE) DESCRIBED FROM
COMMON MYNA (*ACRIDOTHERES TRISTIS*) IN DISTRICT LARKANA, SINDH, PAKISTAN**

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A new trematode *Conspicuum alirazi* n. sp. is described from the Gall bladder of Common myna *Acridotheres tristis* of District Larkana, Sindh, Pakistan. In all, 03 trematodes were recorded; out of them one new species was recorded as *Conspicuum alirazi*. Present trematodes differ from their allies in body shape; size; presence of sub-terminal oral sucker; wider ventral sucker; position of testes and distribution of uterus. On the basis of such morpho-metrical diversification, this species: *Conspicuum alirazi* has been proposed. Authoress dedicated new species in the honor of his brother Mr. Ali Raza Soomro.

PREVALENCE OF TICKS AND TICK BORNE PATHOGENS IN SOUTHERN PUNJAB, PAKISTAN

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Ticks are second to mosquitoes as vectors of a number of human pathogens. So, this study was carried out to check the prevalence of ticks and tick borne diseases in the Southern Punjab, Pakistan. Three districts were selected from the Southern Punjab. The total 30 livestock farms were randomly selected from 03 districts, 10 farms (05 urban and 05 rural) from each district. The collection of ticks was conducted during four seasons of the year 2016 to 2017. The tick samples were taken to research laboratory in clean and dry appropriately labeled plastic bottles. The muslin cloth was used to cover the mouth of these bottles for proper aeration. In the laboratory, the process of preservation was carried out by keeping ticks into 70% methanol. On the basis of morphology the collected ticks were distinguished microscopically with the help of dichotomous key. For molecular studies, ticks from each species were individually used for the extraction of DNA. Extracted DNA of ticks was stored at -20°C. Each tick specimen was screened by PCR for detection of pathogens. The tick pathogens were confirmed by PCR using specific primers. Prevalence of tick and tick-borne pathogens were tested by x2 tests and multiple logistic regressions model which was performed in SPSS 18.0. The prevalence of tick-infected animals was 36.33% (1090/3000) in the Southern Punjab, Pakistan. The total seven tick species were identified i.e. *Hy. anatolicum* 27.14%, *Hy. marginatum* 18.59%, *Hy. dromedarii* 6.91%, *Rh. sanguineus* 15.56%, *Rh. appendiculatus* 11.66%, *B. microplus* 13.86% and *B. decoltratus* 6.25%. *Hy. anatolicum* and *Hy. marginatum* were the most abundant ticks species. The overall prevalence of ticks infestation was significantly different in all animal species. It was observed in buffalo, cows, goats and sheep 35.37 %, 40.87%, 36.00%, 32.57%, respectively. The prevalence of overall evaluations of tick-borne pathogens in the Southern Punjab,

Pakistan was 41.69%. Highest prevalence was found in *Ehrlichia spp* (23.24%) followed by *Theileria spp.* (9.59%), *Anaplasma spp.* (5.9%) and *Babesia spp.* (2.95%). It was concluded that there is wider variety of ticks and tick-borne pathogens is existent in Southern Punjab, Pakistan.

PREVALENCE, HEMATOLOGY AND CHEMOTHERAPY OF GASTROINTESTINAL HELMINTHS IN CAMELS

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Gastrointestinal helminths (GI helminths) are of utmost importance in camels affecting their working potential and productivity. This study was aimed to monitor the status of GI helminths in camels of Cholistan region. This accompanied evaluation of effects exerted by helminthosis on various hematological parameters and comparative therapeutic trails of albendazole and neem leaves against GI helminths in camels. A total of 384 camels were randomly selected in this study. The presence of helminths ova were observed using direct smear method, sedimentation and flotation techniques. An overall prevalence of GI helminths was recorded 66.67% in the study area. Trematodes were found the most prevalent parasite's type followed by nematodes and cestodes. Animals in age range of >10 years were significantly ($p < 0.05$) affected more with nematodes than younger animals (5-10 years age). A non-significant ($p > 0.05$) difference in the prevalence between Berella and Marrecha breeds of camel was observed except for the trematodes where Marrecha breed was affected more (34.67%) than the Berella breed (25.16%), ($p < 0.05$). *Hemonchus spp.* were the most prevalent compared to other nematodes ($p < 0.05$), a non-significant difference was seen in the prevalence among *Fasciola gigantica* and *F. hepatica*, ($p > 0.05$). The only cestode found in this study was *Moniezia expansa* (*M. expansa*). The hematological study found a significant decrease in values of packed cell volume, total erythrocyte count, hemoglobin, and increased in values of total leukocyte count, ($p < 0.05$). The therapeutic trial conducted revealed albendazole as a superior drug over neem leaves (*Azadirachta indica*) in control of GI helminths in camels; however, neem leaves proved to be a successful candidate as an alternative to albendazole.

SEASONAL VARIATION AND FREQUENCY DISTRIBUTION OF ECTOPARASITES IN CATTLE IN DISTRICT BAHAWALPUR

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The aim of this study was to observe the seasonal variations and frequency distribution of different ectoparasites of cattle. This study was performed at District Bahawalpur including 5 tehsiles; Bahawalpur, Yazman mandi, Ahmed Pur East, Khairpur Tamiwali and Hasilpur Punjab, Pakistan. Each tehsil was visited once in each month during 1 year study from January 2016 to December 2016. Total 400 cattle were selected in district Bahawalpur at various livestock farms having different floor pattern (cemented, semi-cemented and non-cemented) and housing system (open, close and semi-close). Different ectoparasites were counted on each selected animal and the mean frequency of different parasites is observed. Ectoparasites were counted directly from the body of each animal from one side and the figure was multiplied with two for whole animal count. The frequency of different ecto parasites fluctuate according to season, climate and temperature.

**PREVALENCE AND CHEMOTHERAPY OF HELMINTH PARASITES IN DEER
(FAMILY CERVIDAE) IN DIFFERENT WILDLIFE PARKS
OF PUNJAB PAKISTAN**

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The present study was aimed to describe the prevalence of helminth parasites in different species of deer (Cervidae family) and investigate the efficacy of different drugs against these parasites. Faecal samples (n=100) were collected from different wildlife parks of Punjab, Pakistan. Coprological examination revealed that 38% samples were positive for GIT parasites, among these point prevalence of cestodes, nematode, trematodes and mixed infection was 12%, 6%, 12% and 8%, respectively. For the chemotherapeutic trails, animals were divided into three groups A, B, and C (10 deer in each group). The group A was treated with Albendazole @ 7.5mg/kg body weight and group B with ivermectin, @ 0.2mg/kg body weight (both orally). Group C was kept as positive control. Faecal samples were collected on the 0 day (pre-treatment), 7th, 14th and 21st day (post-treatment) and revealed that ivermectin was found to be more effective than albendazole as it caused more reduction of the egg count of GIT parasites than Albendazole (P<0.5).

**PREVALENCE OF *FASCIOLA HEPATICA* IN SHEEP, GOATS AND COWS IN
DISTRICT KARAK, KHYBER PAKHTUNKHWA, PAKISTAN**

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Fasciola hepatica (liver fluke) is a type of helminthes under class of trematoda, well known parasitic flatworm in the livers of different herbivorous mammals with cosmopolitan distribution is the main cause of fascioliasis. Fascioliasis is one of the parasitic worm diseases in cattle. Human can also infected by this disease but the ruminants are the main target. It may cause the inflammation of bile duct, gall bladder, gall stone as well as fibrosis. A total of 170 fecal samples were collected from sheep (70), goats (60) and cows (40) in a span of five months i.e. from January to May 2017 from district Karak. Among the cattle sheep showed higher positivity rate (37.5%) than goats (17.6%) and cows (20%). We also investigate that adult cattle were more susceptible for fascioliasis than younger. The present study provides the baseline data for *Fasciola hepatica* in the animals of the study area. Awareness in animals' owners regarding fascioliasis will provide them to control this disease affectively.

**TREATMENT OF MONOGENEAN GILL PARASITES OF SINGLE TAIL GOLDFISH
CARASSIUS AURATUS L. IMPORTED INTO PAKISTAN**

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This study was aimed to investigate the efficacy of three different chemicals: potassium permanganate (KMnO₄), sodium chloride (NaCl) and copper sulphate (CuSO₄) for treatment of monogenean gill parasite *Dactylogyrus* sp. infecting imported single tail goldfish, *Carassius auratus*. The experimental fish (n = 92) was obtained from commercial supplier in Lahore and were examined for gill infections before the experiment.

Three batches of fish (each batch =30 fish) were given bath of KMnO₄ at three different concentrations i.e: 2, 4, 5 mg/l for one hour. Sodium chloride bath was given at the concentration of 5, 7 and 10g/l for a period of 3, 2 and 1.5 hours. Copper sulphate bath was given at concentration of 2 mg/l for 24 and 48 hours. The control groups were kept in separate aquarium and were not given any chemical. The experiments were conducted at 24-25^o C in Fish Disease and Health Management Laboratory. Each batch (n=10) of fish was examined after every treatment and was compared with the prevalence and mean intensity and abundance of parasites before treatment. The result showed that the most effective concentration of KMnO₄ was 5mg/l given for 1hour (infection 16.66%; MI=1.0); 2 mg/l CuSO₄ given for 48 hours removed all the parasites. However, sodium chloride showed least effect on parasites at 10mg/l for 3 hours (infection 100%; MI=12.17). Higher doses of NaCl administered for longer duration may eradicate *Dactylogyrus* sp. in goldfish. The accurate concentration of chemical / drug applied for precise exposure time may be very helpful to treat gill parasites in aquarium fish.

**SOUND PRODUCING APPARATUS AND SONG PATTERNS IN *ACHETA* SP.
(ORTHOPTERA: GRILLIDAE) WITH THEIR IMPORTANCE IN IDENTIFICATION OF
RELATED TAXA KNOWN FROM PAKISTAN**

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In the present work a new species of *Acheta* F. is described from Karachi, Pakistan with special reference to its sound producing apparatus and song patterns with comparison the common house cricket *Acheta domestica* (L.) and recently known species *A. khangpurensis* Khan and Ahmad 2016 found from Pakistan. The morphology of stridulatory file, stridulatory teeth, plectrum, number of teeth, pattern of song, and its frequency were observed to identify the new species. The stridulatory apparatus are important characters in the classification of Ensifera (Desutter-Grandcolas, 2002). Ensiferen acoustic evolution is determined on stridulation organs in Gryllidae (Alexander, 1962; Bailey, 1991; Otte, 1992). Stridulation is considered as a main character to decide its species level. *Acheta* sp. was collected from different areas of Karachi Pakistan at night. Male songs were recorded with the help of Handy recorder. Then recorded, songs were sliced with the help of Audacity 1.3 Beta (Unicoded) software. These sounds were studied and analyzed by using Matlab software. After song recording the specimen was boiled for few minutes to soften the body and detached its right tegmen. Placed the tegmen on stub from its ventral surface. Then the stub was put into a desiccators with Silica gel to dry it. Tegmen was coated with auto coater into JOEL model No. JFC-1500 Japan with gold target, which coated up to 300^o A and scanned with Scanning Electron Microscopy, JOEL Japan Model No. JSM- 6380 A, from Centralized Science Laboratory, University of Karachi, Karachi. Then studied the SEM pictures of the file.

**VIRULENCE OF ENTOMOPATHOGENIC NEMATODES TO HETEROTERMES
INDICOLA (ISOPTERA: RHINOTERMITIDAE) IN THE LABORATORY**

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Termite, *Heterotermes indicola* (Wasmann) is one of the devastating insect pests causing significant losses in different agricultural crops all over the world. Bioefficacy of two Entomopathogenic Nematodes (EPN) species (*Steinernema carpocapsae* & *Heterorhabditis bacteriophora*) in different concentrations i.e 30, 40, 50, 60, 160

individuals per ml of water and control were evaluated against termite workers and soldiers. In control none of the EPN was used. All these seven treatments were replicated 10 times by utilizing completely randomized design. Results revealed that both the EPN @ 160 were found to be most effective and gave significant mortality of termite workers and soldiers followed by EPN 60, 50, 40 and 30. Furthermore, as the concentration and time interval (days) has increased the mortality increased. Based on these results, this study suggested that the EPN has the potential to be incorporated in a biorational management strategy against termites.

SPECIES COMPOSITION AND RELATIVE ABUNDANCE OF SANDBLY IN CUTANEOUS LEISHMANIASIS FOCI, NORTHERN KHYBER PAKHTUNKHWA

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Migration of several million Afghan refugees have led to the spread of cutaneous leishmaniasis (CL) specifically in northern parts of Pakistan. Presence of Afghan refugee camp near Dargai have also resulted in the spread of disease in the area. Present study was designed to determine the species composition of sandflies in Dargai and adjoining areas. Surveys were conducted from March 2015 to April 2017 in five different villages selected on the basis of severity of infection. A total of 15 species of sandflies belonging to two genera were collected from the study area. Genus *Phlebotomus* was represented by three species namely *Ph. pappatasi*, *Ph. sergenti* and *Ph. salehi*, while genus *Sergentomyia* was represented by eleven species i.e. *S. babu*, *S. baghdadis*, *S. bailyi*, *S. grekovi*, *S. mervynae*, *S. dreyfussi turkestanica*, *S. hodgsoni pawlowskyi*, *S. hodgsoni hodgsoni*, *S. fallax afghanica*, *S. indica*, *S. dentate* and *S. theodori*. Both *Ph. pappatasi* and *Ph. sergenti* were collected from the affected areas. *Ph. pappatasi* was found to be more abundant in severely affected area as compared to *Ph. sergenti* pointing towards *Ph. pappatasi* to be the probably vector of CL in the affected area.

EPIDEMIOLOGICAL STUDIES OF GASTROINTESTINAL NEMATODES IN SMALL RUMINANTS OF DISTRICT POONCH, AZAD JAMMU AND KASHMIR

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Gastrointestinal nematodes are internal parasites also known as roundworms. Gastrointestinal nematodes are considered to be most economically important endoparasites of sheep and goats reported to be responsible for causing infection in goats and sheep globally. There is no published data available on the epidemiology of gastrointestinal nematodes in district Poonch, Azad Jammu and Kashmir. Therefore recent study was designed to work out the epidemiology of gastrointestinal nematodes and identification of gastrointestinal nematodes of small ruminants in district Poonch. Study was conducted from 2015 to 2016 in different regions of district Poonch. For this small ruminants (goats and sheep) were selected randomly from the study area. Abomasums were collected from 724 goats and sheep for the observation of gastrointestinal parasites. Direct observation of abomasal parasites were done following standard protocol. Results showed overall prevalence of gastrointestinal parasites was 81% (588/724) in which 87% (397/459) in goats and 72% (191/265) in sheep on the basis of abomasal analysis. Relatively high prevalence of 87% (397/459) nematodes was found in goats than sheep 72% (191/265). Study revealed that high prevalence was found in female goats than male goat. Similarly female sheep suffered more than male sheep. *Haemonchus contortus* was the most prevalent parasite encountered in different regions of district Poonch followed by *Ostertagia* and *Trichostrongylus*. Mixed infection of these all parasites was also found. High prevalence was found in spring season. Goats had large dominance of *H. contortus* than sheep. Region wise result showed high predominance of gastrointestinal parasites in Rawalakot region followed by Hajira, Abbaspur and Paniola region. Least prevalence of gastrointestinal parasites was observed in Thorar region in both male and female sheep and goats.

STUDY ON SKIN AND GILLS PARASITES OF NILE TILAPIA FROM KARACHI COAST**Baby Tooba and Bushra Khalil***Department of Zoology, Jinnah University for Women, 5-C Nazimabad Karachi*

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The protozoan parasites of *Oodinium sp.*, *Trichodina sp.*, *Dermocystidium sp.*, and *Volvox sp.* were observed on skin and *Oodinium sp.*, *Trichodina sp.*, *Dermocystidium sp.*, *Volvox sp.* and *Microsporidium sp.* were found on the gills of fish *Nile tilapia*. *Oodinium sp.* intensity was 34.48%, *Trichodina sp.* intensity was 21.83%, *Dermocystidium sp.* intensity was 22.98%, *Volvox sp.* intensity was 20.68%. *Oodinium sp.* intensity was 20.43%, *Trichodina sp.* intensity was 32.25%, *Dermocystidium sp.* intensity was 15.05%, *Volvox sp.* intensity was 23.65%, *Microsporidium sp.* intensity was 8.60%. The samples were collected from Karachi fish harbor and from fish market Musa colony. 150 fishes named *Nile tilapia*, for the identification of protozoan parasites were collected. 200 Permanent slides were prepared with the help of direct smear of skin and gills. These parasites carried harmful effects on *Nile tilapia* fish skin and gills and cause severe diseases and resulted in economic losses or mortality, treatment expenses, growth reduction during and after outbreak of disease.

PREVALENCE OF ECHINOSTOME (TREMATODA) IN MICROCHIROPTERA (PIPISTRELLUS PIPISTRELLUS / PIPISTRELLUS PYGAEMUS) BATS OF PAKISTAN**Muhammad Mazhar Ayaz and Muhammad Mudasser Nazir***Department of Pathobiology, Bahauddin Zakariya University, Multan*

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The bats are considered for vector of various viruses and parasites. The parasitological survey was conducted on bats of microchiroptera the common pipistrelle (*Pipistrellus pipistrellus/ Pipistrellus pygaemus*) that is a small pipistrelle bat which very largely extends across most of Europe, North Africa, and Southwestern Asia including Pakistan. Ten freshly naturally died bats were investigated for the prevalence of parasites including trematodes located in the intestinal tract at Department of Pathobiology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan. Among them 7 bats (70%) of them were found infested with trematodes including Echinostome spp. which were dominant in small intestine. The common pipistrelle is the smallest bat found in Pakistan which shows morphometry ranging 3.5–5.2 cm (1.4–2.0 inch) long along the head-and-body, with the tail adding 2.3–3.6 cm (0.91–1.42 inch). The body mass ranges from 3.5 to 8.5 gm (0.12 to 0.30 oz), with the wing-span ranging from 18 to 25 cm (7.1 to 9.8 inch). Its brown fur is variable in tone from grayish brown to bronze brown.

MOLECULAR IDENTIFICATION OF LARVAE OF TREMATODE DICROCOELIUM DENDRITICUM N BRACHYLAIMA SP. FROM INTERMEDIATE HOSTS (GASTROPODS) IN UZBEKISTAN**A.E.Kuchboev¹, M.H.Egamberdiev²**¹*Institute of zoology Uzbekistan Academy of Sciences, str. Bogishamol, 232B, 100125, Tashkent, Uzbekistan,*²*Namangan State University, Namangan, Uzbekistan*

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Dicrocoeliosis is a widespread parasitic disease in grazing animals worldwide. The infection is common in Europe, Asia, North Africa, and America, where the local conditions are suitable for particular species of terrestrial gastropods and ants as intermediate hosts. The infection is also common in sheep, goats, cattle, equids, rabbits Uzbekistan. The disease causes severe economic losses, in terms of milk and meat production, due to liver function impairment. It can be fatal on rare occasions. The species of genus *Brachylaima* Dujardin, 1843 is a member of family of Brachylaimidae and comprises the primary endoparasites of endothermic vertebrates, including birds, mammals, and humans. Terrestrial gastropods are required as the first

and second intermediate hosts in the life cycle of *Brachylaima* sp. First intermediate hosts have sporocysts and cercariae, and second intermediate hosts have metacercariae of *Brachylaima* sp. Infection occurs when the definitive hosts consume raw gastropods containing metacercariae. In this regard, it is very important to accurately identify species belonging to larvae of trematodes parasitizing in the gastropods of Uzbekistan. The use of classical morphological methods to identify larvae of trematodes is difficult, and requires high qualification of a specialist. The definition of taxa below the genus with the help of morphological methods of studying only the larval stage is often completely impossible. Therefore, the purpose of this study is the molecular identification of larvae of trematodes of gastropods in the natural and synanthropic zones of the Fergana Valley. The material for the study was gastropods collected in the Namangan region of the Fergana Valley during the autumn period of 2016. Gastropods were collected on the banks, elephants hill, under stones, in ravines, grass, on the stems of plants, near shrubbery, on pasture areas. To identify the larval stages of trematodes in terrestrial gastropods, a compression method was used, then compression of the hepatopancreas of gastropods. The liver was isolated from the dissection needles, and then it was ground on a slide and under a cover glass in a slightly compressed form was examined under a ML 2000 microscope (Meiji). We analyzed single cercariae of trematodes isolated from infected liver (hepatopancreas) from two species gastropods of *A. regeliana* (Bakharstan village of Kasansay district) and *X. candacharica* (vicinity of the reservoir of Kasansay) and one specimen of *P. sogdiana* (foothills of Govasoy of Chust district of Namangan region. In this case, genomic DNA was isolated from individual cercariae of trematode. In the experiment, a Qiamp DNA kit (Qiagen, Hilden, Germany). When determining the sequence of D2 28S rDNA sites using PCR (iCycler iQ Real Time PCR BIORAD, USA), 1 µl of each C2 primer (5'-GAAAAGAACTTTGRAR-3') and D2 (5'-TCCGTGTTTCAAGACGGG-3'), 1 µl DNA with a master mix GoTaq Green (Promega Corp., USA) in a volume of 25 µl reaction [94°C for 03 minutes, 40 x (94°C for 30 seconds, 40°C 01 minutes, 68°C for 01 minutes), 68°C for 10 minutes]. Electrophoresis of the amplified DNA fragments was carried out in a 1.5% agarose gel and purified using a GenElute™ PCR Clean-Up Kit (Sigma-Aldrich). The PCR products were sequenced from both directions with the primers used in the amplification by the center of Euro fins Sequencing (Japan). One of the most effective markers for identifying the species of larvae of trematodes was the 28S rDNA sequence. As a result of amplification of the partial sequence of 28S rDNA, we obtained fragments from five samples of larvae of trematodes derived from gastropods (534-590 bp for *A. regeliana*, 558-566 bp for *X. candacharica* and 592 bp - *P. sogdiana*). Alignment of the first four sequences of 28S rDNA with Genbank data shows a 99% similarity to the species of *Dicrocoelium dendriticum*. The next specimens of larvae from gastropods studied belonged to the species *Brachylaima* sp. with a 98% similarity to the species of the genus *Brachylaima*. A phylogenetic tree was constructed using a 28S sequence from other representatives of the families Dicrocoeliidae and Brachylaimidae, taken from the GenBank database. The tree was built using the maximum likelihood and closest neighbors (Maximum Likelihood and Neighbor joining) method using the MEGA version 6 software package. The results PCR data showed that the samples studied larvae from intermediate hosts gastropods in Uzbekistan belonged to two species of trematodes of *Dicrocoelium dendriticum* и *Brachylaima* sp.

DETECTION OF MULTIPLE ANTHELMINTIC RESISTANCE IN GOATS DISTRICT PALLANDRI, AZAD JAMMU KASHMIR

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Goats are vital part of livestock and agriculture sector which help in national economic development throughout the world. Goats are managed for leather, milk, and meat purposes. The possible production of goats is largely affected by the presence of multiple anthelmintic resistance in them. The current study has been planned to detect multiple anthelmintic resistance in gastrointestinal nematodes of goats managed at various parts of Pallandri Azad Jammu and Kashmir. *In vivo* evolution of multiple anthelmintic resistance were done by using Faecal Egg Count Reduction Test, Mean faecal egg count, percentage reduction and 95 percent confidence level was calculated

by the help of the formula suggested by the World Association for the Advancement of Veterinary Parasitology for evaluating multiple anthelmintic resistance. Two goat herds were classified into four different groups for treatments and each group contain 10 goats: Albendazole, Levamisole, Mixture of Albendazole and Levamisole and a control group treated with water. The percentage decrease in fecal egg count (95 percent confidence intervals) for Levamisole, Albendazole and mixture of both flocks I were found 91.0 %, 91.0 % and 95.0 % and for Flock II were 90.0 %, 91.0 % and 95.0 % (Efficacy in percent). Results of Faecal Egg Count Reduction Test show a significant difference ($P < 0.05$) on pre treatment and post treatment with treatment groups as contrary to control group of treated with water. The results revealed that the presence of multiple anthelmintic resistance in experimental flocks. Two herds were detected for resistance compared to the Albendazole; two were found resistant against the levamisole, sensitive for resistance against mixture of both Albendazole and Levamisole. Control efficacy test results revealed that presence of gastrointestinal nematodes of species *Haemonchus contortus*, *Ostertagia circumcincta* and *Strongyloides* species.

**DESCRIPTION OF NEW TREMATODE *PSILOCHASMUS PLATYRHYNCHOSI* N.SP.
(TREMATODE: PSILOCHASMIDAE) IN MALLARD *ANAS PLATYRHYNCHOS*
(ANSERIFORMES: ANATIDAE) OF SINDH, PAKISTAN**

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In result of ongoing helminthological studies of mallard *Anas platyrhynchos* of Kambar Shahdadkot District of Sindh Province, Pakistan, a total of 20 birds were captured from different localities. During examination of gut contents and visceral organs, 65 specimens of *Psilochasmus platyrhynchos* n.sp. collected from intestine of the host bird. *Psilochasmus platyrhynchos* n.sp. differs from its close allies in body shape and size, distribution of vitellaria which is densely scattered in hind body, shape of cirrus sac presence of seminal receptacle size and shape of testes and ovary and size of eggs. On the basis of these diagnostic differences, a new species *Psilochasmus platyrhynchos* is proposed.

PREVALENCE OF SCABIES IN DISTRICT DADU

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Scabies is a parasitic skin infestation caused by the mite *Sarcoptes Scabies* Var hominis. It occurs worldwide, and as many as 300 million people may be affected. Scabies infestation occurs in people of both sexes and all the ages irrespective of ethnic group or socioeconomic status, being most common in conditions of over crowding, poverty, and poor hygiene. In development countries, the infestation rates occur similarly across all age groups, while in developed countries, higher susceptibility to infestation occurs in the age range of preschool children to adolescents. Scabies is endemic in the developing world, yet it is not recognized as a disease of public health priority in most developing countries. The World Health Organization has recently added scabies to its list of Neglected Tropical diseases. Several studies show scabies is a problem in the developing world, including Nigeria. Scabies is transmitted by close person to person contact which facilitates the transfer of the mite from one person to another. The mite burrows under the skin causing type IV hypersensitivity reactions with intense pruritus which is worst at night. The resultant skin lesions from hypersensitivity and scratching are prone to secondary infection with bacteria namely group A streptococci and staphylococcus aureus. Scabies secondarily infected with bacteria gives rise to a range of complications such as folliculitis, impetigo, cellulitis, abscesses, septicemia, glomerulonephritis, rheumatic fever, rheumatic heart disease and even death. According to DHIS Repot for the year of 2016-17 in civil Hospital Dadu 1448 cases of scabies were registered and scabies found mostly in Khairpur Nathan Shah (K.N) Shah, Johi Kachoo Distract Dadu.

SECTION - V**FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY,
MARINE BIOLOGY**

- 1. ECOLOGY AND ENVIRONMENTAL POLLUTION**
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1. ECOLOGY AND ENVIRONMENTAL POLLUTION

FOOD WEB STATUS OF SOIL MACRO-INVERTEBRATES IN CULTURED AND NON-CULTURED SOIL

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The current study was designed to record "Food web status of soil macro-invertebrates in cultured and non-cultured soil" under ecological zone in Punjab (Faisalabad). From both fields, overall 1591 specimens were recorded from both fields, maximum population was recorded from cultured soil 62.54 % (N = 995) and least population was recorded from non-cultured soil 37.46% (N = 596). From cultured soil, entire population was recorded pertaining 13 orders, 36 families, 50 genera and 59 species. Where in from non-cultured soil, population of soil macro-invertebrates was recorded pertaining to 11 orders, 29 families, 40 genera and 44 species. The biomass in case of cultured soil maximum biomass was recorded during 11th sampling (28.64 ± 10.62) at 43°C and 21% humidity, and least biomass was recorded during 3rd sampling (2.73 ± 7.70) at 13°C and 84% humidity. In case of non-cultured soil, maximum biomass was recorded during 11th sampling (19.1 ± 7.71) at 43°C and 21% humidity, and least biomass was recorded during 3rd sampling (3.58 ± 3.26) at 22°C and 67% humidity. Overall relative abundance of each species was variable from each other and between each field. In from cultured soil, it was accessed that *Trochulushispidus* (Hygromiidae) was recorded as an extraordinary contributing species with relative abundance of 17.39% (N = 173), and in case of non-cultured soil *Trochulushispidus* (Hygromiidae), was recorded as an extraordinary contributing species with relative abundance of 23.83% (N = 142). Diversity (H') was recorded highest (2.2488) from cultured soil as compared to non-cultured soil (1.1490). Highest evenness value was again recorded from cultured soil (0.0830) and least from non-cultured soil (0.0537). Dominance was recorded highest from cultured soil (1.0830) and least from non-cultured soil (1.0537). Richness (R) was recorded maximum in cultured soil (16.3135) and least from non-cultured soil (12.7699). Analysis of Variance (ANOVA) among both soils (cultured and non-cultured) showed non-significance results (F=0.64; P=0.4331). t-test enlisted that in overall strength, soil macro-invertebrate was existed non-significantly in both soils (t-value= 2.85; P-value= 0.0146). Linear Regression confirmed that structural community as well as taxa composition were differing significantly among both soils (F=202.99; P≤ 0.001).

ENVIRONMENTAL CHANGES AND THEIR EFFECTS ON HERDRO WETLAND SINDH

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Wetland have a played an important role in life. Herdro lake natural brackish water lake, 10 km northwest to Thatta and 85 KM east of Karachi at about 7 KM from Makli to jang Shani road in Thatta district Sindh province. The Herdro Lake is important of resident and migratory birds regularly visit in different seasons. One of the important function of wetland is to provide a habitat for water birds. The lake an important wintery and summer migratory water fowls includes Flamingo, Ducks, Coots, Glossy ibis, Waders, Gull and tern. The present study physiochemical of water quality like water quality like conductivity, TDS, Turbidity, Salinity, PH, Alkalinity, Carbon dixide, Phosphates, Total Hardness, BOD, COD and population statistics of avifauna has been estimated. The surveys were conducted for duration of 12 month August 2016 to July 2017. The area was regularly visit in all season during above period and birds fauna were record by the help of binocular the birds fauna were identified by T.J Robert (1991) and Sonbe and Usui (1993). The total of water birds recorded during study period was 55 species. The habitat is degraded due to misuses of natural resources. Decline the birds population indicates the environmental pollution.

A COMPREHENSIVE ANALYTICAL STUDY OF HABITAT DEGRADATION AND ITS IMPACT ON AQUATIC ANIMALS IN SINDH, PAKISTAN

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The present study was proposed to evaluate quality of aquatic habitats for animals such as amphibians and fishes in Larkana district of Sindh Province. Present investigation consisted of field surveys and laboratory work conducted monthly from year 2011 to 2013. While exploring the study area, animal species including *Pethia ticto*, *Cirrhinus cirrhosis*, *Labeo calbasu*, *Labeo rohita* and *Catla catla* under Class Pieces will recorded, whereas within the Class Amphibia, three species including *Hoplobatrachus tigerinus*, *Euphyctis cyanophlyctis* and *Bufo stomaticus* were discovered. Water quality of altogether 26 habitats was analyzed via study of physico-chemical parameters: pH, electric conductivity (EC), total dissolved solids (TDS), total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl), sulfate (SO₄), phosphate (PO₄), nitrite (NO₂), nitrate (NO₃), carbon dioxide (CO₂) and potassium (K) using analytical equipment and methodology. Mean value and standard deviation (\pm) of all parameters was measured as followed: pH 7.8 ± 0.7 , EC 2506.3 ± 1139.1 , TDS 1712.1 ± 581.2 , T. Hard 534.2 ± 170.5 , T. Alk 284.4 ± 65.9 , Cl 423.2 ± 97.7 , SO₄ 451.8 ± 122.1 , PO₄ 429.4 ± 94.3 , NO₂ 4.5 ± 2.4 , NO₃ 7.2 ± 3.1 , CO₂ 18.5 ± 3.7 and K 74.3 ± 9.8 . The value of all parameters (except pH and CO₂) was measured extremely high beyond the tolerance of animals in question. This study recorded wide-ranging water quality problems which may affect populations of fishes and amphibians badly as their eggs and larvae are highly sensitive to environmental degradation.

SUSTAINABLE MANAGEMENT PLAN FOR CONSERVATION OF WETLAND NATURAL RESOURCES

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Wetlands resources are affected by various human and natural aspects. Instead of providing numerous ecological roles for fauna, flora and community, these environment purifiers are facing lots of threats. Wetland management needs a multidisciplinary procedure that incorporates the mechanical, financial, ecological, legal and social features. The current study was done to design a sustainable management for conservation of Mangla Dam natural resources. The PHRIA (Participatory Human Resource Interaction Appraisals) was used for collection of information regarding demography, management of natural resource, village economy system, education, human resource interaction and biodiversity knowledge. Interviews were conducted from 392 persons among them 158 were females and 234 were males. A total of 163 plant species, 13 species of mammals, 188 species of birds, 42 species of fish, reptiles (n=12) and amphibians (n=2) were recorded at site by point count method. It was found that study site was susceptible to various threats like deforestation, poverty, illiteracy, grazing, illegal hunting, unsustainable fisheries, poor wildlife protection law enforcement, pollution and community hatred. Hunting of migratory birds along with fauna was momentous at dam, during three years of surveys 539 cases of illegal hunting were reported and calculated hunting index was 14.7. On the basis of research findings three zones were identified that needed collaborative effort of community, concerned department and all stakeholders for conservation of natural resources of Mangla Dam. These included wetland preservation zone, sensible utilization zone and community Zone. For success of intended plan implementation of some actions like catchment area conservation, water management, conservation of biodiversity, Declaration as RAMSAR site and improvement of local community livelihood conditions are very vital. These findings and proposed sustainable management plan will also be useful for other wetlands of Pakistan and will open new corridor for future research plans for conservation of Pakistan biodiversity.

STATUS OF AMPHIBIAN HABITATS IN SUBDIVISIONS OF DISTRICT LARKANA “BAKRANI AND RATO DERO”, SINDH, PAKISTAN

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The present study was carried out as a consecutive investigation of earlier exploration of amphibian diversity in Taluka Bakrani and Rato Dero where three amphibian species: *Hoplobatrachus tigerinus*, *Euphyllotis cyanophyllotis* and *Bufo stomaticus* were recorded. Present research based on physico-chemical study of amphibian habitats was conducted from March to October in year 2013 using analytical instruments and standard procedures. The parameters selected for present investigation included pH, electric conductivity (EC), total dissolved solids (TDS), total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl), sulfate (SO₄), phosphate (PO₄), nitrite (NO₂), nitrate (NO₃), carbon dioxide (CO₂) and potassium (K). The results showed value of all the parameters in subdivision “Bakrani” as followed: pH (7.8±0.7), EC uS/cm (2617.1±1089.8), TDS mg/L (1815.7±641.8), T-Hard mg/L (603.0±221.4), T-Alk mg/L (318.7±53.5), Cl mg/L (454.6±113.4), SO₄ mg/L (492.4±148.0), PO₄ mg/L (420.1±86.1), NO₂ mg/L (7.2±4.3), NO₃ mg/L (9.0±3.4), CO₂ mg/L (18.9±3.7) and K mg/L (73.4±10.1). Meanwhile in subdivision “Rato Dero” the value of water quality parameters was noted as pH (8.0±0.8), EC uS/cm (2948.6±1649.1), TDS mg/L (1825.0±714.6), T-Hard mg/L (538.5±173.8), T-Alk mg/L (271.9±74.9), Cl mg/L (423.8±91.3), SO₄ mg/L (448.5±114.4), PO₄ mg/L (445.2±101.8), NO₂ mg/L (4.6±1.6), NO₃ mg/L (7.8±2.8), CO₂ mg/L (18.6±3.1) and K mg/L (75.6±9.2). High values of physico-chemical parameters implied massive rate of pollution in both study areas. Present study revealed hazardous level of entire parameters into all aquatic habitats which may have adverse effects on delicate creatures in question and may cause them to decline nearby. This alarming status of amphibian habitats may be treated urgently for the survival of amphibians of the study area.

DEGRADATION ISSUES OF AMPHIBIAN HABITATS IN TALUKA DOKRI (A SUBDIVISION OF DISTRICT LARKANA), SINDH PAKISTAN

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A water quality study was carried out in amphibian habitats at taluka Dokri. From seven agricultural ponds (permanently inhabited by amphibians), water samples were collected and analyzed for the determination of physico-chemical parameters including pH, electric conductivity (EC), total dissolved solids (TDS), total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl), sulfate (SO₄), phosphate (PO₄), nitrite (NO₂), nitrate (NO₃), carbon dioxide (CO₂) and potassium (K) using material and methods of analytical grade from March to October 2013. Present study recorded value of parameters too high to support amphibian survival in entire study area where only pH value (7.6±0.8) and CO₂ (mg/L) value (19.8±3.9) were measured within normal range, however value of other parameters including EC (uS/cm): 2030.6±531.5, TDS (mg/L): 1504.2±337.5, T-Hard (mg/L): 464.2±108.2, T-Alk (mg/L): 271.5±59.8, Cl (mg/L): 378.9±74.5, SO₄ (mg/L): 398.1±75.2, PO₄ (mg/L): 395.3±82.7, NO₂ (mg/L): 3.9±1.6, NO₃ (mg/L): 5.6±1.6 and K (mg/L): 73.7±9.3 was recorded extremely high up to adverse level. Variation in value of parameters was recorded every month with maximum upsurge in July, while minimum value of all parameters was noted in October, but it was duly recorded that even minimum range of all parameters was still too high to meet favorable criteria. Present investigation confirmed the existence of massive pollution into all aquatic habitats in “Dokri subdivision” which may affect amphibian fauna negatively.

FOOD WEB STATUS OF SOIL MACRO-INVERTEBRATES IN CULTURED AND NON-CULTURED SOIL

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The current study was designed to record "Food web status of soil macro-invertebrates in cultured and non-cultured soil" under ecological zone of Faisalabad (Punjab). From both fields, overall 1591 specimens were recorded from both fields. From cultured soil, entire population was recorded pertaining 13 orders, 36 families, 50 genera and 59 species. Wherein from non-cultured soil, population of soil macro-invertebrates was recorded pertaining to 11 orders, 29 families, 40 genera and 44 species. The biomass in case of cultured soil maximum biomass was recorded during 11th sampling (28.64 ± 10.62) at 43°C and 21% humidity, and least biomass was recorded during 3rd sampling (2.73 ± 7.70) at 13°C and 84% humidity. In case of non-cultured soil, maximum biomass was recorded during 11th sampling (19.1 ± 7.71) at 43°C and 21% humidity, and least biomass was recorded during 3rd sampling (3.58 ± 3.26) at 22°C and 67% humidity. Overall relative abundance of each species was variable from each other and between each field. In from cultured soil, it was accessed that *Trochulus hispidus* (Hygromiidae) was recorded as an extraordinary contributing species with relative abundance of 17.39% (N = 173), and in case of non-cultured soil *Trochulus hispidus* (Hygromiidae), was recorded as an extraordinary contributing species with relative abundance of 23.83% (N = 142). Diversity (H') was recorded highest (2.2488) from cultured soil as compared to non-cultured soil (1.1490). Highest evenness value was again recorded from cultured soil (0.0830) and least from non-cultured soil (0.0537). Dominance was recorded highest from cultured soil (1.0830) and least from non-cultured soil (1.0537). Richness (R) was recorded maximum in cultured soil (16.3135) and least from non-cultured soil (12.7699). Analysis of Variance (ANOVA) among both soils (cultured and non-cultured) showed non-significance results (F=0.64; P=0.4331).

COMPARISON OF SOIL MACRO-FAUNAL DIVERSITY IN WHEAT (*TRITUM AESTIVUM* L.) FIELDS BETWEEN DIFFERENT ZONES

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The present research work was conducted to record "Comparison of macro faunal diversity in wheat (*Tritium aestivum* L.) fields between different zones" in Punjab province for future strategies. it was observed that soil macro fauna is bio indicator toward aggravation of soil environment and state of the dirt nourishment web. From both areas, overall 194 specimens were recorded from restricted and open wheat fields as a whole, but variably, maximum population was recorded from restricted fields 58.76% (N=114) and lest population was recorded from open field 41.24% (N=80). Taxa composition also recorded momentarily different from both areas e.g. from restricted fields, entire population was recorded pertaining to 9 orders, 19 families, 24 genera, and 27 species. Wherein from open fields population of soil macro fauna was recorded pertaining to 7 orders, 16 families, 23 genera, and 26 species. Population means from restricted fields, species abundance was recorded maximum during 3rd sampling (2.91 ± 0.96) at 15 °C and 80% humidity, and biomass was recorded utmost and equal during 1th and 6th sampling (0.75 ± 0.57) and (0.68 ± 0.62) at 23 and 24°C; 55 and 46 % humidity, respectively, however least biomass was recorded during 5th sampling (0.4 ± 0.81) at 23 °C and 70% humidity. In case of open fields, maximum population was recorded during 9th sampling (1.69 ± 0.62) at 31°C and 32% humidity, and biomass was recorded utmost equal during 6th and 7th sampling (0.63 ± 0.13) at 21 and 22.8 °C; 46% and 41% humidity, respectively. However last biomass was recorded during 1st sampling (0.041 ± 0.54) at 26.11 °C and 79 % humidity. Overall relative abundance of each species was variable from each other and between each field. in restricted field, *Gonocephalum missellum* (Tenebrionidae) was recorded highest

contributing species with relative abundance of 10.53% (N=12) and from open wheat fields *Gonocephalum simplex* (Tenebrionidae) was recorded highest contributing species with relative abundance of 13.75 % (N=11). In restricted wheat field from total of 10 recorded orders, 09 orders were recorded with relative abundance was recorded highest for order Coleoptera (Beetles) 39.47% (N=45), followed by Dermaptera (Earwig) 12.28% (N=14), Isopoda (Woodlice), Araneae (Spiders), Pulmonata (Snails). In case of open wheat fields, all the orders (7 orders) were recorded and relative abundance was recorded extraordinary for order Coleoptera (beetles) 51.25 % (N=41), isopoda (Woodlice) 17.50% (N =14). However, richness (R) was recorded maximum in restricted field (4.4649) and least from open field (3.1067). Analysis of Variance (ANOVA) was made, after completing the analysis it was observed that population mean of recorded taxa among both fields (Restricted and Open) showed non-significance results (F=0.36; P=0.5578). Linear regression confirmed that structural community as well as taxa composition were differing significantly among both fields (F=67.47; P ≤ 0.001).

ANTHROPOGENIC ACTIVITIES AND WATER QUALITY: A CASE OF RIVER SATLUJ

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Pakistan exhibits a larger fresh water reservoir Ecosystem. Out of them River Satluj is the main tributary of the Indus river. Length of river Satluj in Pakistan encompasses 329 miles. It is one of the three rivers whose water management has been given to India under Indus Water Treaty. Pakistan with the help of World Bank, has made water reservoirs and link canals to use the river bed of Satluj. The main objective of study was to find out Water Quality Parameters during the present study seasonally. Overall seven factors were selected for the current study; which were temperature, pH, dissolved oxygen, total dissolved solids, total alkalinity and total hardness. All the water quality parameters under investigation revealed their presence in permissible limits as prescribed by National and International quality guidelines (except dissolved oxygen) and fit for aquatic fauna. Dissolved oxygen remained (4 ppm in summer) and (4.5 ppm in winter) at SZ-1. It might be due the less water discharge and the presence of municipal and industrial wastes at Sulemanki Barrage (Study Zone-1) of the study area.

TROPHIC GUILDS AND RELATIVE ABUNDANCE OF SOIL MACRO-FAUNA IN WHEAT (*TRITICUMAESTIVUM*) FIELDS OVER SPACE AND TIME

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The present research was conducted to record the "Trophic guilds and relative abundance of soil macrofauna in wheat (*Triticumaestivum*) fields over space and time" during the session 2015-2016 under the ecological conditions of district Narowal. From both fields, overall 240 specimens were recorded. As a whole, nearly equal population was recorded from both fields but variably, maximum population was recorded from Boundary of wheat fields 52.50% (N=126) and least population was recorded from center of wheat fields 47.50% (N=114). Taxa composition also recorded momentarily different from both fields e.g. from center of wheat fields, entire population was recorded pertaining to 11 orders, 27 families, 3 classes and 27 species. Wherein from boundary of wheat fields, population of soil macro-fauna was recorded pertaining to 11 orders, 24 families, 4 classes and 37 species. Wherein from center of wheat fields, species abundance was recorded maximum during 1st, 2nd, 3rd and 5th sampling (5 species) at 26.11°C, 20°C, 15°C and at 17°C temperature and

79%, 64%, 63% and 51% humidity, and least abundance was recorded during 4th and 9th sampling, 3 species at 6°C and 31°C; while at 86% and 32% humidity. In case of boundary of wheat fields, maximum population was recorded during 2nd sampling (8 species) at 20°C and 64% humidity, and least abundance was recorded during 4th sampling 4 species at 6°C and 86% humidity. In case of center of wheat fields, maximum biomass was recorded during 3rd sampling (3.34 ± 1.41) at 15°C and 63% humidity, and least biomass was recorded during 6th and 9th sampling (0.44 ± 0.64) at 21°C and 31°C and 46% humidity and 32% respectively. In case of boundary of wheat fields, maximum biomass was recorded during 1st sampling (3.78 ± 1.49) at 26.11°C and 79% humidity, and least biomass was recorded during 6th sampling (0.42 ± 0.88) at 17°C and 46% humidity. In center of wheat fields, from total of 12 recorded orders, 11 orders were recorded, and relative abundance was recorded extraordinary for order Coleoptera (Beetles) 43.86% (N=50), and in case of boundary of wheat fields, from total of 12 orders, 11 orders were recorded, and relative abundance was recorded extraordinary for order Coleoptera (Beetles) 47.62% (N=60). Diversity (H') was recorded highest (2.0315) from boundary of wheat fields as compared to center of wheat fields (2.0285). Highest evenness value was again recorded from boundary of wheat fields (0.0150) and least from center of wheat fields (0.0139). Dominance was recorded highest from boundary of wheat fields (1.0150) and least from center of wheat fields (1.0139). Richness (R) was recorded maximum in boundary of wheat fields (11.3277) and least from center of wheat fields (9.1769). Analysis of variance (ANOVA) among both sites (center and boundary of wheat fields) showed non-significance results (F=0.03; P=0.8702).

A STRONG AND RELIABLE ECOLOGICAL STRESS MARKER FOR LIVING BOVIDS

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Bovids are of high economic importance for Pakistan as well as many other countries as a potential source of meat and milk. Ecological problems faced by the bovids can add up a lot in decrease of their reproductive potential and growth rate. In Pakistan most bovids are semi domestic in nature so environmental stress can be estimated well in these animals if a reliable marker is present within their body. Enamel Hypoplasia is a dental defect cause by the depletion of ameloblast, as ameloblasts are highly sensitive cells so any environmental stress faced by these semi domestic bovids during their tooth development can be observed as a linear horizontal mark of enamel hypoplasia on their tooth enamel. Current study carried out on goat, sheep, cow and buffalo teeth showed that enamel hypoplasia can be a reliable and permanent tool for estimation of comparative level of stress in all these four representatives of family Bovidae. This tool can be a good contributor in improving the health status of this taxon in the country.

STUDY THE QUALITY CHARACTERISTICS OF POTABLE WATER IN KARACHI AND ITS IMPACT ON HUMAN HEALTH

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In Pakistan, the water pollution is the main cause of diarrheal diseases and deaths among their people because about 62% urban and 84% rural population do not treat their drinking water. The present study based on evaluate and compare the drinking water quality among the 18 towns of Karachi city with WHO standards and its related diseases among the people of Karachi. During the present study total 90 samples were collected from Feb 2017-Dec 2017, the samples were analyzed in laboratory physical and chemical parameters of drinking water like pH, salinity, dissolved oxygen, electrical conductivity, chlorine, Nitrate, Ammonia, total alkalinity and total hardness were examined. The percentage level of the drinking water coloration, taste, smell and its related diseases among people were also determine. The results revealed that in whole 18 towns of the Karachi city, as not 100% colorless, tasteless and odorless drinking water is supplied to the consumers. The

laboratory findings disclosed that the concentration level of electrical conductivity of water samples in most of the towns like Baldia, Gulberg, Jamshed, Kemari, Korangi, Landhi, Mair, New Karachi, Orangi and Shah Faisal towns, the concentration level of nitrate in Gulberg town, total alkalinity level in all towns excluding only in Malir town and total hardness level in Korangi, Liaquatabad and Saddar towns were exceeded above the limits specified by WHO; while the chlorine concentration level in all drinking water samples was below the specified limit, however, in Gulberg, Korangi, Landhi, Liaquatabad, Lyari, New Karachi, North Nazimabad, Saddar and S.I.T.E towns, the chlorine level was totally absent in water samples, other drinking water parameters like pH, salinity, dissolved oxygen and ammonia were within the limit in all water samples of the whole 18 towns. The risk of both diarrhea and skin and eye irritation among people was increased as compared to other diseases because of the absence of chlorine and exceeded level of electrical conductivity and total alkalinity was examined in drinking water samples of most of the towns of the Karachi city. While the lower risk of other diseases like heart disease, Methemoglobinemia and kidney stone among people was examined because in water samples of the few towns, the exceeded level of total alkalinity, nitrate and total hardness was estimated. Thus, present study mentioned that the drinking water quality condition in most of the towns of Karachi city was deteriorated and it is not fit for human consumption. For the provision of pure and clean drinking water to the citizens of Karachi city, the study suggested that the drinking water sources should be regularly monitored and also more filtration plants should be established near the major drinking water sources of Karachi city through the government authorities.

ECOLOGICAL STATUS OF SPINY TAILED LIZARD (*Saara hardwickii*) FROM LESSER CHOLISTAN DESERT, BAHAWALPUR, PUNJAB

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Current study was conducted to collect data on ecological observation of spiny tailed lizard (*Saara hardwickii*) in the area of lesser Cholistan desert, Bahawalpur. The density of lizard recorded was based on presence of active burrows. The number of subadults (48%) was higher in the population followed by juveniles (31%) and adults (21%). This species has territorial behavior and showed wide fluctuations with varying temperature. During normal hot days the lizards were found to be active in the early hours of the morning. During breeding season, lizards remain close to their burrows. They remain alert while feeding, run very fast, and back to their respective burrows on the slightest hint of disturbance, mainly from predators. The lizard eating its own shed skin observed during study was earlier reported in other lizard which is to fulfill nutrient requirements. In Cholistan, these lizards are often illegally collected and sold in various parts for folk medicine. It is kept in captivity by the cruel practice of dislocating the backbone. Detail study is required on ecology of spiny tailed lizard for its conservation in Cholistan desert, one of main area of its distribution in Pakistan.

TRADITIONAL ZOOTHERAPEUTICAL STUDY IN THE CHOLISTAN DESERT OF BAHAWALPUR

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The present study describes the traditional knowledge related to the use of different ethno-zoological based medicine and animal derived products used as medicines by the inhabitants of the Cholistan desert of

Bahawalpur (Pakistan). A field survey was conducted from 2006 to 2007 by performing interviews through structured questionnaire with selected respondents, who provided information regarding use of animals and their products in folk medicine. A total of 20 animal species were recorded and they are used for different ethnomedicinal purposes, including asthma, general debility, sexual debility, skin diseases, paralysis, arthritis, tuberculosis, etc. The zootherapeutic knowledge was based on both domestic animals as well as wild animals. On the base of this communication we could suggest that this kind of neglected knowledge should be included into strategies of conservation and management of faunistic resources. Further studies are required for experimental validation to investigate the bio-active compounds in these animals' raw material.

EFFECT OF LEATHER TANNERIES EFFLUENT ON SURFACE AND GROUND WATER QUALITY OF DISTRICT KASUR

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Leather export has a major role in the economy of Pakistan, contributing 5% of total GDP. Tanneries for the leather production use chemical like sulfuric acid, chrome, sodium, sulfates and bisulfates for tanning process which are associated with degrading the surface as well as ground water quality. The current study investigates surface and ground water quality of city Kasur, being the main hub of tanneries. Water samples were collected in triplicates from six sampling points; two tanneries, two surface water sites and two ground water sites. Physical (PH, Conductivity, Temperature) and Chemical parameters (sodium, potassium, Calcium, phosphorus, nitrate, sulfates heavy metals I.e. Chromium, lead and arsenic) were examined using pH, conductivity meter and atomic absorption spectrophotometer respectively. Results indicates acidic nature of all the samples which is may be due to the excessive use of sulfuric acid and formic acid. Conductivity of all the samples were 2times, higher from the standard limits which is may be due to the excess use of sodium, sulfates and bisulfates. Prevailing situation calls for the attention by the Federal and provincial agencies for the proper implementation of legislations to improve the water quality of kasur city.

2. FRESHWATER BIOLOGY AND FISHERIES

IMPROVING FLOATING POTENTIAL OF AQUA-FEEDS

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Ever increasing population, dwindling natural water resources and highly polluted nature of the water reservoirs are the major driving forces for rapid development of aquaculture in many countries. To decrease the cost of fish feed plant origin protein sources are used. Six diets based on different protein levels employing sunflower, canola, maize gluten and rice polish. The best diet on the basis of retention time was proceeded for further study. Ten diets based on different soaking times were prepared and then evaluated for floatability. Retention time of prepared diets increased with increasing soaking time. Among the studied feed ingredients; the floatability and retention time indicated rice polish as best candidate to enhance floatability of aqua feed. The diet based on sunflower meal, canola meal, maize gluten and rice polish had 23.33%, 25%, 25%, 26.67%, 30.00% and 43.33% floatabilities at 10%, 15%, 20%, 25%, 30% and 35% protein levels, respectively. The soaking time of diet played significant role in improving the floating potential of aqua feeds. Applications of these findings can improve feed conversion efficiencies.

GROWTH EVALUATION OF TILAPIA (*Oreochromis niloticus*) REARED IN FULLY CONTROL SYSTEM

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Tilapia (*Oreochromis niloticus*) was reared in a fully control system to evaluated the growth performance. A trial of the experiment was conducted for the period of one year (May to April) in cemented; pond having an area of 40,000 ft² and filled up to one feet with water. Hence, 100,000 fingerlings of tilapia (*Oreochromis niloticus*) with mean initial weight of 10±2.1 gram were stocked within a volume of 40,000ft³. The fish were fed with plant origin formulated pelleted consisting 19% protein, prepared form rice by product. All the essential water quality parameters including dissolved oxygen, temperature, pH, TDS, conductivity, salinity and NH₃ were measured and maintained in the required range. Fish were harvested with a mean final weight of 520 ± 80.2 gm; with average mean final weight gain of 510 ±78.1. During the study survival rate was recorded 99% with specific growth rate (SGR) 0.47% per day. In result a production of 51,480 Kgs were obtained from 40,000ft³. Hence, tilapia can be cultured in a control system to produce maximum production by utilizing a minimum area.

IDENTIFICATION OF TIME OF GONADAL DIFFERENTIATION IN CATLA (*CATLA CATLA*) AND GRASS CARP (*CTENOPHARYNGODON IDELLA*)

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Time of sex differentiation has been identified in two major carps; catla (*Catla catla*) and grass carp (*Ctenopharyngodon idella*). Developmental process of gonads in these species was studied from fertilized egg stage till completion of sex differentiation. Identification of this time is important to find out an appropriate time

for sex reversal treatment to produce monosex culture at commercial level thus eliminating the growth rate differences between both sexes. Sex differentiation was at 840°dph (degree days post-hatch) and 1215°dph in catla and grass carp respectively.

BIOAVAILABILITY OF NUTRIENTS BY SMALL SCALE HOUSEHOLD AQUACULTURE TO COMBAT MALNUTRITION IN RURAL AREAS OF SINDH, PAKISTAN

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Small scale household aquaculture has been one of the chief nutritional sources in many traditional rural communities of Sindh and is a great approach to fulfill the nutritional requirement of animal protein of rural populations, who suffer from vitamin and mineral deficiencies. A study was designed to portray the nutritional contribution of rural aquaculture to combat deficiencies of micronutrients among rural populations of District Thatta, Sindh. Programmed surveys were conducted in priority areas on the basis of having high potential for fish farming for the collection of samples to review bioavailability of vitamin A, calcium, iron, and zinc contents in cultured fish species. Small scale aquaculture of indigenous fish species could contribute significantly to the intakes of macro and micronutrients in rural populations and was proved a promising strategy to improve nutritional status of inhabitants.

LENGTH-WEIGHT RELATIONSHIPS OF *CYPRINUS CARPIO* (GULFAM) FROM THE INDUS RIVER AT CHASHMA LAKE, DISTRICT MIANWALI, PUNJAB, PAKISTAN

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An attempt was made to study on LWR, LLR and condition factor (K) of exotic carp, *Cyprinus carpio* (L., 1758) at Chashma Lake district Mianwali, Punjab, Pakistan. Samples of 43 fishes range in size of 31.00 cm to 60.80 cm were taken to estimate the LWR and LLR. *Cyprinus carpio* was weighed in gram. The length of fish were ranged in size from 31.00 to 60.80 cm with a mean of 43.50 (± 1.98) in TL and from 25.00 to 50.00 cm with a mean range of 35.20 (± 1.87) in SL. The regressions results for LWR were found highly significant ($r = 0.943$; $P < 0.001$) with coefficients of determination, $r^2 = 0.889$ showing highly significant correlation between logs transformed data of TL and W. Slope value (b) of *C. carpio* was found 3.01, which is very close to 3.00, hence representing an isometric growth pattern. In LLR, the SL, BG, DFL, PvFL and AFB were found isometric ($b=1$) with TL. HL, PtFL, PtFB, PvFB, AFL, CFL and CFW showed negative allometric growth, while DFB showed positive allometric growth with TL in *C. carpio*. Condition factor (K) remained insignificant with total length and body weight.

ICHTHYOFAUNAL DIVERSITY AND ASSEMBLAGE STRUCTURE IN RELATION TO THE HABITAT CHARACTERISTICS AROUND KARACHI POWER PLANT

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Present study is based on the data sets acquired from demersal trawl net operated in different seasons of the year (2016-17) near Karachi Power Plant. Over eight thousand individuals, 119 species belonging to the 47

families were collected during entire fishery survey programme. Overall catch trends demonstrates that finfish (clupeids, mullids, sciaenids) biomass was higher than shellfish (crabs, shrimps, cephalopods). Average finfish biomass of 10.4, 30.65, 81.2 & 68.3 (Kg) was fished in May, September, December and March respectively. Highest number of species encountered belongs to the family Carangidae, Scianidae and Apogonidae. Rarefaction counters was applied to evaluate frequency of occurrence of each individual segregate by rarefaction method showed that individuals of most of species caught were < 300, whereas *Upeneus supravittatus* comprises of > 1000 and *Nemipterus japonicus* > 3000 were frequently appeared in the catches. Principal component analysis (PCA) was applied on twenty five species in relation to the habitat characteristics water temperature, salinity, pH, depth and oxygen were analyzed. Biomass and catch per unit effort (CPUE) estimates are stock indicators for management of fishery resource, highest CPUE calculated in December and the lowest in March 2017.

HISTOLOGICAL CHANGES IN STOMACH OF FISH *PLECTORHINCHUS CINCTUS* (TEM. & SCHL.) OF KARACHI COAST

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The present study deals with the histological changes in the stomach of fish *Plectorhinchus cinctus* (Tem. & Schl.) caused by trematode parasite. Trematodes were collected from infected *Plectorhinchus cinctus*, the effect of trematode causing damage wall was obvious. The effects of trematode on *Plectorhinchus cinctus* have not been reported previously. Histological studies of stomach were carried out by standard techniques. The cellular changes were observed both macroscopically and microscopically. Macroscopic observation revealed edema, inflammation and mucous secretion in stomach tissue. While microscopic observation revealed hypertrophy, hyperplasia and at some portions of tissue fibrosis were common. The cellular changes were studied using microtomy technique.

FRESHWATER FISH DIVERSITY AND THEIR CONSERVATION STATUS IN SOUTHERN PUNJAB, PAKISTAN

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Understanding fish diversity and its distribution patterns are principal issues for scientists and managers concerned with the extinction processes of fresh water fish species. The aim of current review is to compare the relevant literature describing the diversity, ecology and current population conservation status of freshwater fish of the Southern Punjab, in Pakistan. Peer reviewed papers, published during 1976 to 2017, describing freshwater fish diversity at the study site, were selected. Out of four (04) set criteria, two criteria were regarding diversity and distribution of freshwater fish, whereas the other two were related to conservation status and their ecological importance. In total, 82 freshwater fish species belonging to 17 families has been reported at the study site. It was observed that, out of these reported families, most dominant families were Cyprinidae (53.7%), Sisoridae (7.3%), and Bagridae and Channidae 4.9% each. Conservation status of the studied freshwater fish fauna of Southern Punjab indicated 73% of the total species as least concern, 16% species were not evaluated, 6% were near threatened, 4% were found vulnerable, whereas 1% of the total observed species as data deficient. Published record showed that fresh water fish population is continuously decreasing due to overexploitation, illegal fishing activities, alteration in water flow through diversion and damming. The freshwater fish population has also been reduced intensely due to many factors including pollution, habitat degradation, and overexploitation. In conclusion, certain measures including establishment of nature reserve, simulated proliferation and releasing are needed to conserve fish diversity in the area. Habitat restoration should be given consideration for the restoration of diversity of fish in their natural environment. A complete survey to evaluate the current diversity and conservation status of different freshwater fish species is, furthermore, highly recommended at the study site.

**EFFECT OF STOCKING DENSITY ON GROWTH PARAMETERS OF CATFISH
RITA RITA IN CEMENTED CISTERNS**

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Study the effect of stocking density on various growth parameters of catfish *Rita rita* (Hamilton) in cemented cisterns was initiated during June to August 2016. Three stocking densities such as 10, 20 and 30 fish/1.25m² cemented cisterns were assigned to the experimental fish to observe suitable density for better growth and survival rate. The net weight gain of individual fish in treatment II was higher (16.4 g) than those of Treatment I (11.7 g) and (8.8 g) in treatment III respectively. The survival and specific growth rates were also found highest in treatment II (100% and 0.48) followed by treatment I (90% and 0.36). While significantly (p<0.05) lowest survival rate and SGR was recorded (80% and 0.31) from treatment III. It was concluded that the suitable stocking density 20 fish/m²(treatment II) exhibited significantly higher growth of *Rita rita* in cemented cisterns among the treatment

**HISTOLOGICAL STUDY OF SEX DIFFERENTIATION IN BIGHEAD CARP
(*HYPOPHthalmichthys nobilis*), AND SILVER CARP (*HYPOPHthalmichthys molitrix*)**

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Time of sex differentiation has been identified in two major commercial carps; bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). Histological differentiation of germ cells has not been previously studied in these species. Developmental process of gonads in these species was studied from fertilized egg stage till completion of sex differentiation. Identification of this time is important to find out an appropriate time for sex reversal treatment to produce monosex culture at commercial level thus eliminating the growth rate differences between both sexes. Sex differentiation was observed at 784°dph (degree days post-hatch) (28 days post-hatch) and 786°dph (28 days post-hatch) in bighead carp and silver carp, respectively under natural climatic conditions in Lahore.

**FEEDING HABITS AND DIET COMPOSITION OF *SALMO TRUTTA FARIO* (THE BROWN
TROUT) IN UPPER PARTS OF RIVER SWAT, PAKISTAN**

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This study was aimed to determine the prey selection and feeding habits of *Salmo trutta fario* (the brown trout) in upper parts of river Swat, Pakistan from March 2016 to April 2017. During the study period a total of 185 guts of the brown trout were examined. Twenty four stomachs were found empty. Feeding intensity was represented by fullness index (FI), changing with various length groups and season. The most important food item of brown trout were brachycentridae, blepharocera, hydropsychidae and ephemereillaspp. Kruskal Wallis test were applied on feeding intensity the H statistic is H=8.13 with DF=3 and P=0.043. Here the P (0.043) is <

alpha (-0.05). The regression between N and IRI is $N = 35.2 + 22.1 \% IRI$ 1% change in IRI there is 22 in prey number. Pearson correlation of total length (mm) and fish body weight (gm.) $r = 0.976$, P-Value = 0.024. The stomach fullness shows that feeding intensity was recorded higher from March to May. A total of 2289 preys including; trichoptera, hydropsychidae, brachycentridae, diptera, blepharocera, ephemeral, chironomida, honey bees, grass hopper, locust, trout eggs, trochanters, plant tissues, stones were recovered from the gut contents of brown trout. According to index of relative importance (IRI%) four preys represents major components of the diet. The highest IRI% was recorded in brachycentridae (39.38%), followed by blepharocera (13.23%), hydropsychidae (10.76%), ephemeral spp (8.28%). The relationship between IRI and FO is ($r=0.556$) which is moderate positive correlation with coefficient of determination ($r^2=0.309136$). It was concluded that brown trout is the principal species of family salmonidae inhabited in cool water bodies of the study areas. Gut contents of brown trout were analyzed on monthly basis. Present study will help in the development of an artificial diet for better growth in culture system for the members of the family salmonidae.

ENVIRONMENTAL MONITORING OF RIVER CHENAB THROUGH FISH LIVER AND KIDNEY HISTOPATHOLOGY EVALUATION

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The objective of this study was to highlight the effects of domestic wastes and industrial effluents on water bodies and organisms especially fish. Histopathological alterations were observed in the Liver and Kidney of *Cirrhinus mrigala* collected from the polluted site of River Chenab, Cheniot District, Punjab, after receiving drain water near Wara Thatta Muhammad Shah (31° 32' 57" N, 72° 32' 6" E). The water samples were collected from selected sites along the river and Chakbandi drain and analyzed for heavy metals. Heavy metals concentrations like of Pb (3.54±0.17 mg/L), Cu (4.786±0.01 mg/L) and Ni (1.32±0.008 mg/L) were found above the WHO permissible limit. *Cirrhinus mrigala* collected from river downstream site were compared with control fish collected from Government Fish Hatchery, Faisalabad. Major histopathological alterations in liver included hypertrophy of hepatocytes, cytoplasmic degeneration, and hemolysis between hepatocytes, and degeneration. Kidney showed severe damage like the dilation of both Bowman's space and the glomerulus were observed from kidney lesions. Whereas in case of control *Cirrhinus mrigala* specimen of liver and kidney revealed normal architecture. This study accentuates that environmental monitoring via use of fish biomarker (histopathology) could prove their worth to evaluate effects of environmental contaminants that have likely to affect human health via food chain.

POPULATION DYNAMICS OF REBA CARP *CIRRHINUS REBA* FROM INDUS RIVER, SUKKUR, SINDH, PAKISTAN

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This study was conducted to assess the current stock of *Cirrhinus reba* Suhni (Reba carp) from Indus River, Sukkur, Sindh, Pakistan for first time, because stock assessment provides the baseline information to the fisheries managers to make better strategic plans for sustainable exploitation of the commercially important fish species. The length-frequency data was collected from October 2015 to March 2016. A total of 463 pairs of length-weight were measured. The minimum length recorded as 13cm and maximum length as 29cm with average length of 17cm. While minimum weight was measured as 15g and maximum as 224g with an average

weight of 67g. The estimated values of length-weight relationship were $a = 0.037$, $b = 2.557$ and $R^2 = 0.817$. The calculated von Bertalanffy growth function parameters using ELEFAN method in FISAT computer package was $L_{\infty} = 29.40$ cm and $k = 0.240 \text{ year}^{-1}$. The estimated values of growth performance index were $\phi' = 2.317 \text{ year}^{-1}$. The estimated rate of total mortality Z applying the length-converted catch curve analysis method was $Z = 0.630 \text{ year}^{-1}$, natural mortality M was estimated as: 0.617 year^{-1} at temperature of 21°C while the rates of fishing mortality F was calculated as: 0.012 year^{-1} . Hence exploitation ratio ($E = F/Z$) was calculated as 0.019 year^{-1} . When biological reference points F_{opt} for *C. reba* was equal to M (0.617) current fishing mortality rate of 0.012 was smaller than the target biological reference points. While the MSY was recorded as; 8.144 which were higher than the current catch of 0.031 tons year^{-1} . This study concluded that the *C. reba* confronting poor health though the stocks exists at sustainable level in Indus River Sukkur, Sindh, Pakistan.

ESTIMATION OF LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF *LABEO ROHITA* UNDER CORN BASED INGREDIENTS WITH VARYING PROTEIN LEVELS

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A comparative study was conducted for 120 days to evaluate the effect of corn as a carbohydrate source both in gelatinized and non-gelatinized form at different protein levels i.e. 30%, 35% and 40% on growth and condition factor (K) of *Labeo rohita*. Fingerlings were distributed randomly to aquaria of each having dimensions 90L×30W×45H (cm) with 29 L water capacity. Two replicate were used per treatment with the stocking of fifty fingerlings in each replica. Six test diets were prepared i.e. T₁: G, 30% CP; T₂: NG, 30% CP; T₃: G, 35% CP; T₄: NG, 35% CP; T₅: G, 40% CP and T₆: NG, 40% CP. After acclimatization of one week, fingerlings were fed with test diets twice daily to satiation at 4% of live wet body weight and the fingerlings were transferred to other aquaria fortnightly for weight and length measurement. At the termination of experiment, among all the diets, T₆: NG, 40% CP showed the highest value of K as 11.86 followed by T₂: NG, 30% CP (10.75), T₁: G, 30% CP (10.65), T₄: NG, 35% CP (10.41), T₅: G, 40% CP (10.10) and minimum value of K was showed by T₃: G, 35% CP (9.95) respectively. The exponential equation from length weight relationship showed that overall mean condition factor did not significantly deviate from the value of 1.0, which depicts high precision of the regression model and showed that fish were in good condition thus justifying the dimensional equality of their growth pattern.

ENVIRONMENTAL AND PHARMACEUTICAL APPLICATIONS OF FISH SCALES

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The present investigation was based on "Environmental and Pharmaceutical Applications of Fish Scale" for the detections of the mineral composition, particularly calcium content of fish scales of *Pomadasys maculatus*. It has been achieved by the technique of *Scanning Electron Microscopy* and *Energy Dispersive Spectroscopy*. The obtained outcome revealed that the concentration of calcium mineral (Ca) is too high followed by $\text{P} > \text{O} > \text{C} > \text{Al} > \text{Si}$ in the fish scales. In this study, it has been observed that the calcium and other minerals found in the fish scales can be utilize as bio-waste products in the scientific society. As calcium is the most important component of body, which is mostly found in the form of hydroxyapatite that gives strength to our teeth and bones, supports in proper cellular functioning and helps in the transmission of electrochemical nerve impulses. When the actual amount of calcium is become deficient in body, then it causes multiple bone disorders and interrupts in various body functions. By collaborating with the pharmacists, the calcium obtained

from the fish scales can be further processed and used in the manufacture of medicines or calcium supplements. In addition, by using this bio-waste product (fish scales) can influence our environment as healthy and clean.

COMPARATIVE IMMUNITY OF INTERGENERIC HYBRIDS AND PARENTAL SPECIES *CATLA CATLA* AND *LABEO ROHITA*

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Hybridization is common in fish for attaining best characteristics of parental species. Here an attempt has been made to compare the immunity of two closely related cyprinids *Catla catla*, *Labeo rohita* and their intergeneric reciprocal hybrids by studying their immunological indices, i.e., Total Protein (mg mL⁻¹), Lysozyme (µg mL⁻¹), Igm (mg mL⁻¹), Phagocytic Activity %, Phagocytic index and Respiratory Burst. Results indicated higher immunity of hybrids as compared to parental species. However, interspecies comparison revealed higher immunity of *L. rohita* while *C. catla* ♀ × *L. rohita* ♂ hybrid showed higher values of immunological indices as compared to hybrid produced by reverse cross (*C. catla* ♂ × *L. rohita* ♀). The results of present study may signify the importance of selection of cross in hybridization for improving hybrid vigor.

EFFECT OF PHOTOPERIOD ON GENERAL MORPHOLOGY AND PHYSIOLOGY OF ROHU *LABEO ROHITA* (HAMILTON, 1822)

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Photoperiod regulate the biological rhythm of many organisms by effecting, physiological, biochemical and behavioral processes. Thus change in photoperiod alters these processes and affect the welfare of fish. The current study investigated the effect of three different photoperiods, 14L/10D, 10L/14D and 6L/18D on growth, general health and physiology of fingerlings of Rohu (*Labeo rohita*). A 90 days experiment was conducted in a completely randomized manner in replicate of three under laboratory condition. Fingerlings exposed to prolonged photoperiod showed significantly higher ($P < 0.05$) growth rate, feed conversion efficiency and survival (%) as compared to other photoperiods. The complete blood count (CBC) an indicator of health status also indicated significantly higher ($P < 0.05$) values of HB, MCV (fL), MCH, LYM%, RBCs and MCHC (%) in a group of fish reared under 18L/6D. However, fingerlings exposed to prolonged scotophase (6L/18D) showed significantly ($P < 0.05$) higher cortisol and glucose levels, darker body colorations with deep yellowish and darkest spots on the body, damaging of scales as well as narrow body structure. The results indicate the negative impact of prolonged scotophase and suggest culturing this species in areas of Pakistan having long day photoperiod.

GROWTH OF SCALES IN A FRESHWATER FISH, *CHANNA STRIATA* FROM KARACHI, PAKISTAN

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This study deals with the growth of scales in a freshwater fish, *Channa striata* from Karachi, Pakistan. For this purpose, samples were collected from local fish market Bermi colony-Korangi, Karachi. The lateral line

scales were selected for scale collection. Four to six scales were collected from the selected body region and clean with diluted NaOH. Clean scales were placed between two microscope-glass slides. Each slide was studied under microscope and selected scale parameters were measured or counted. For study of scale growth, total length of scale (TLS), width of scale (WDS), distance from focus to outer margin (Rs) and radii (RDS) on scales were studied. During this study, the results of regression analysis between total length of fish and different parameters of scales were obtained as:

$TL = 116 + 23.1 \text{ TLS}$; $TL = 157 + 18.3 \text{ WDS}$; $TL = 165 + 26.4 \text{ Rs}$; $TL = 205 + 2.40 \text{ RDS}$

The correlation between total length of fish and different parameters of scales were observed as $TL \text{ vs } TLS=0.614$, $TL \text{ vs } WDS=0.675$, $TL \text{ vs } Rs=0.564$ and $TL \text{ vs } RDS=0.285$.

FISH DIVERSITY OF LOWER SWAT CANAL IN DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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The fish faunal study was carried out to unfold the diversity of freshwater fish fauna in Lower Swat Canal of flows throughout the District Swabi, KP, Pakistan. The sites selected for the collection of fish species were; Neknam, Yar Hussain, and Jaganat. The process of collection was done from January to March 2017 on every 14th and 28th of the month. A total number of 10 species were collected under the process of legal mean and after identification process they were named as *Labeo rohita*, *Onchorhynchus mossambicus*, *Channa gachwa*, *Mystus blakry*, *Pontius sarana*, *Mastacembelus armatus*, *Heteropneustes fossilis*, *Crossochilus diplocheilus*, *Pontius sotori*, *Ompok pabda*. The collected species after identification were belonged to 4 orders and 7 families. The process of collection was done by using of small nets, by using of scoop nets, by using of basket nets, and by using of specialized hooks. From the above it is cleared that the water body of Lower Swat Canal is good for the culturing of fishes. And family *Cyprinidae* was dominant in the related spot. It is also noted that the main reason of the reduction in relates water body is due to the high water pollution and waste extracts of industries and houses. Illegal hunting of fishes must be avoided to get rid of the problem.

DETECTION OF INTERSPECIFIC HYBRIDIZATION AMONG CHINESE CARPS BY USING MOLECULAR MARKERS

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Inadvertent hybridization in fish has triggered serious ecological instabilities such as loss of distinctive genetic diversity and decline in species fitness. Molecular markers have been established as useful tool to assess variety of ecological perspective for observing the genetic integrity of natural populations. The present research work was conducted to illustrate the degree of potential hybridization and genetic introgression events occurring in Chinese carps using morphometric and molecular analysis. Total 30 samples of each fish were collected from selected public fish hatcheries of Punjab (i.e. Faisalabad, Jhang, and Farooqabad) then instantly preserved for transport. Morphometric analysis was carried out with the help of identification key while for genetic characterization, a panel of molecular markers viz. RAPDs and microsatellites were used. For RAPDs and microsatellite based detection of hybrids, genomic DNA was isolated and subjected to PCR amplification of target loci for subsequent genotyping. Five universal and five species specific primers i.e. Hmo11, Hmo13, Hmo25, Hmo33 and Hmo34 were prepared to assess genetic variations. The data obtained from these studies were subjected to statistical analysis using various softwares i.e. FSTAT, GENEPop, TFPGA to estimate population differentiation and genetic diversity. Multi-dimensional analysis were conducted to check population

genetic variation and hybrid detection (via GENETIX ver. 4.05). Such analysis demonstrated extent of genetic variations in each population. The values of FIS ranged between 0.2452 to 0.463 while in some cases, the negative values of FIS points out the occurrence of hybridization/crossbreeding in subject populations. The study shows that the hatchery populations of Chinese carps are undergoing genetic deterioration due to poor breeding practices leading to inbreeding and hybridization.

EFFECT OF SOAKING ON FLOTABILITY OF FISH FEED INGREDIENTS

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Albeit substitution of animal origin protein sources in fish feed have necessitated with the use of plant-derived protein feedstuffs. Though, problems of anti-nutritional factors in plant based protein ingredients (sunflower meal, canola meal, maize gluten (30%) and rice polish) have limited their usage and assimilation into fish feed formulation but soaking is very important to increase their nutritional profile. A low cost fish feed preparation process based on 35% protein level was optimized regarding the functional properties of pellets by Central Composite Design (CCD) of Response Surface Methodology (RSM). The independent variables used were substrate and water ratio (X_1), temperature (X_2), soaking time (X_3), and pH (X_4). Twenty six experiments under different conditions were conducted to optimize different soaking times (6, 12, 18, 24 and 30 hrs.), temperatures (4, 15, 26, 37 and 48°C), pH (2, 4, 6, 8, and 10) and substrate to water ratio (1:1, 1:3, 1:5, 1:7 and 1:9) which were then used to determine different parameters that are responsible for floating. Pellet properties observed for each set of these variables were moisture content, floatability, water absorption index (WAI) and water solubility index (WSI). The moisture content ranged from 0.33% to 0.62% and floatability of fish diet under different conditions varied from 10% to 46.67%. Water absorption index (WAI) altered from 2.11g to 2.83g and the water solubility index (WSI) varied from 12.8% to 26.48%. These results depict that soaking conditions of diet plays a significant role in improving the floating potential of aqua feed.

DIGESTIBILITY OF FOOD WASTES AS FEED INGREDIENTS FED TO SILVER CARP

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Apparent digestibility coefficients (ADCs) for crude protein, crude fat and carbohydrates of three agri-wastes (pea peels, potato peels and wheat straw) were determined for freshwater fish silver carp. The feed ingredients were analyzed for proximate composition prior to the formulation of reference and test diets. One reference and three test diets were prepared containing 35 % protein by linear formulation method. The fish specimens were fed at the rate of 2 % body weight twice a day on reference (control) diet and test diets composed of 700 g/kg reference diet and 300 g/kg test ingredient. Chromic oxide (Cr_2O_3) was used as an inert indicator. After two hours of the feeding session, feces were collected using suction and pressing method. Water quality parameters (temperature, pH and dissolved oxygen) were monitored on daily basis through digital meters. The collected fecal material from each aquarium were dried and stored for chemical analysis. After the termination of experiments, each fish specimen of each aquarium were weighed and then dissected. Different portion of intestine were taken and stored for digestive enzyme analysis. The apparent digestibility of protein for pea peel ($83.84 \pm 1.00\%$) was higher than potato peels ($65.6 \pm 0.43\%$) and wheat straw ($65.99 \pm 0.27\%$). The apparent lipid digestibility and apparent carbohydrates digestibility were high for pea peels up to $74.36 \pm 1.13\%$ and $86.63 \pm 0.27\%$, respectively. Final weight gain (%), specific growth rate (%) was higher for diet 1 with lower feed conversion ratio. Amylase and protease activity were higher at anterior portion and minimum at posterior portion of intestine. Knowledge of ADC values for these ingredients allow feed producers to develop nutritionally balanced, low-cost feed formulations for this species.

**EFFECT OF CORN BASED FEED INGREDIENTS WITH VARYING PROTEIN LEVELS
ON GROWTH PERFORMANCE OF *LABEO ROHITA***

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A feeding trial was carried out for 120 days to observe the effect of corn as a carbohydrate source both in gelatinized and non-gelatinized form at different protein levels i.e. 30%, 35% and 40% on performance of growth and morphometric characteristics in *Labeo rohita*. Fingerlings were distributed randomly to aquaria of each having dimensions 90L×30W×45H (cm) with 29 L water capacity. Two replicate were used per treatment with the stocking of fifty fingerlings in each replica. Six test diets were prepared i.e. T₁: G, 30% CP; T₂: NG, 30% CP; T₃: G, 35% CP; T₄: NG, 35% CP; T₅: G, 40% CP and T₆: NG, 40% CP. After acclimatization of one week, fingerlings were fed with test diets twice daily to satiation at 4% of live wet body weight and the fingerlings were transferred to other aquaria fortnightly for weight and length measurement. At the termination of experiment, highest average body weight gain and total length was exhibited T₄ (NG, 35% CP) which were statistically non-significant. These findings concluded that gelatinized corn at 35% protein level proved as a promising fish feed ingredient being more efficiently utilized and showed better growth results in *Labeo rohita*.

**EFFECT OF FREEZING ON ETHER EXTRACT AND PROTEIN CONTENT OF ROHU
(*LABEO ROHITA*) AND MORI (*CIRRHINUS MRIGALA*)**

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Fish is a source of protein rich in essential amino acids and has important elements for the maintenance of healthy body. Generally, wastage of fish through spoilage has been estimated to range from 18% to 30% in developing nations. This spoilage may be due to chemical, enzymatic or microbial activities. Freezing is the main method of processing fish for human consumption and the most used to reduce biochemical changes that occur during storage. Fish is purchased from fish hatchery Sialkot. Fresh samples of all trials are analyzed to determine fat and protein contents. The remaining samples were refrigerated at two different temperatures (-2°C to -16°C). The ether extract and protein contents were analyzed after 15, 30 and 45 days from the start of experiment. It was observed that freezing is an effective technique for fish preservation as least degradation was observed if protein and fat contents of both fish.

**STUDIES ON WATER QUALITY ANALYSIS AND FISH BIODIVERSITY OF
DADU CANAL FROM SINDH, PAKISTAN**

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Dadu canal is a tributary emanates from Sukkur Barrage Indus River and ends at district Dadu, Sindh. It provides habitat to various kinds of fish species which include Wallago attu (jarrko), cat fish (khaggo), suhnni, Notopterus notopterus (Ganddan) and so on. Amongst them cat fish and jarrko are two most populous species in the canal. More than 35 different kinds of species are collected from two regularities which are located at village Massu sahar and village Shahpanjo Sultan in taluka Mehar District Dadu. In water analysis the temperature of canal alters with the change in annual climatic weather. In summer the temperature of water of bottom is 22°C while its surface temperature is 24°C. While in winter its bottom temperature is 28 to 30°C and its surface temperature is 22°C. Moreover, its PH is 7.03, salinity 1000 mg/c TDS and its conductivity 150 to 500 u/cm. This is the brief sketch of the two sites of Dadu canal.

FUNGAL INFECTION IN AN IMPORTED ORNAMENTAL FISH RED TIGER OSCAR, *ASTRONOTUS OCELLATUS*

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The present study was conducted to investigate fungal and parasitic infestation in imported red tiger oscar fish, *Astronotus ocellatus*. Thirty fish were purchased from local pet shop in Lahore. Out of thirty fish 24 fish were studied for fungal infection and six were studied for parasitic infestation. The mean body weight and mean total length of the experimental fish was 6.6 ± 0.5 g and 16.51 ± 1.2 cm respectively. Clinical signs shown by infected fishes were; pop eye, eroded fins especially caudal fin, severe mucous secretion and darkening of skin. Material from the infected site of fish was taken and inoculated on two agar media; Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). The inoculated plates were incubated for 5-7 days at $28-30^{\circ}$ C. A variety of colored fungal colonies such as: black, grey, white, pink, brown, off white, orange yellow and green appeared on agar plates. The material from agar plates was taken and slides were prepared and stained by 0.05% trypanblue in lactophenole. Six fungal genera; *Aspergillus*, *Mucor*, *Alternaria*, *Fusarium*, *Blastomyces* and *Penicillium* were isolated from different organs of fish such as: eye, gills, skin, operculum, dorsal fin, pectoral fin, pelvic fin caudal fin and anal fin. Overall infection was 91.6%. The maximum infection was shown by *Aspergillus* (73.33%) and minimum was shown by *Mucor* (4%). The anterior portion (head, eye, gills, operculum, and pectoral fin) of the fish was the most affected area than the posterior (anal fin, caudal fin, pelvic fin and dorsal fin). Pure culture of *Aspergillus*, *Mucor*, *Alternaria* and *Fusarium* were prepared from original cultured plates of these fungal genera and plates of pure culture showed same morphology as in already prepared plates. The number of parasites in each examined fish was counted and identified. Examined fish for parasitic infestation showed different type of parasites such as: *Dactylogyrus* sp., *Gyrodactylus* sp., *Argulus* sp. and *Ichthyophthirius multifiliis*. The mean intensity and infection of each parasite was *Dactylogyrus* sp., (527.75, 66.7%), *Gyrodactylus* sp., (559.5, 33.33%), *Argulus* sp. (6, 16.7%) and *I. multifiliis* (1.5, 33.33%) respectively. *Dactylogyrus* was most commonly found on gill lamellae and tips of the filaments. *Gyrodactylus* and *Argulus* were found in the skin of the fish. *Ichthyophthirius multifiliis* was found in the gills. There is need to improve the maintenance of aquarium, water quality and fish feed used in aquarium shops. The strict regulations on import of ornamental fish may be implemented.

FUNGAL INFECTION IN AN IMPORTED ORNAMENTAL FRESHWATER ANGELFISH, *PTEROPHYLLUM SCALARE*

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The aim of present study was to investigate incidence of fungal and parasitic infestation in ornamental angelfish *Pterophyllum scalare*. The fish (n=30) was purchased from local market in Lahore. The mean body weight and total length of 30 fish was 2.24 ± 0.31 g and 5.07 ± 0.32 cm respectively. Twenty six fish were observed for mycological infections. The clinical picture of *Pterophyllum scalare* showed that caudal, anal and dorsal fins were eroded. The scales were slightly eroded. The material from diseased areas of fish (gill, skin, operculum, eyes, dorsal, anal, pelvic, pectoral and caudal fin) were inoculated on two types of culture media Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA). The fungus was allowed to grow for 6-7 days and incubated at the temperature of $28-30^{\circ}$ C. Fungal colonies of different colors: black, green, grey, white, brown, orange-yellow and off-white were observed. The material from agar plates was taken and slides were prepared and stained by 0.05 % trypanblue in lactophenole. A total of 239 culture plates were prepared. Four fungal genera: *Aspergillus* sp., *Alternaria* sp., *Blastomyces* sp., and *Mucor* sp. were identified. *Aspergillus* sp. was the most prevalent fungus found in 20 examined fish. *Alternaria* sp. and *Aspergillus* sp. were found in association. The overall percentage

infection of *Aspergillus* sp. was 80.1%, *Mucor* sp. was very low 1.2 % and *Alternaria* sp. and *Blastomyces* sp. were 7.4% and 6.1% respectively. The infection was maximum in skin (18.33%) and caudal fin (15%), dorsal fin (12%) and gill (11.7%). The minimum infection was observed in anal fin (5%). Four fish were examined for parasitic infestations. All examined fish were having gill infection by *Dactylogyrus*. Two fish were heavily infected with 75 and 100 parasites respectively. Mean intensity of *Dactylogyrus* sp. was 61.25 and prevalence was 100%. In this study, fungal and parasitic infections were observed in imported angelfish *Pterophyllum scalare*. It is suggested that import of diseased ornamental fish must be controlled.

THE STUDY OF ICHTHYOFAUNAL DIVERSITY OF LOWER BADRAI STREAM IN DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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The present study was conducted from January 2017 to April 2017 in order to explore the ichthyofaunal diversity of lower Badrai Stream in District Swabi, Khyber Pakhtunkhwa, Pakistan. During the said study, fifteen freshwater fish species were identified. These fish species were taxonomically embodied to four orders including Siluriformes, Perciformes, Cypriniformes, Synbranchiformes, and to nine families including Cyprinidae, Mastacembilidae, Cichlidae, Heteropneustidae, Channidae, Siluridae, Ambicidae, Sisoridae and Bagridae. Family Cyprinidae was the most dominant family by representing five fish species consisting *Barilius pakistanicus*, *Schizothorax esocinus*, *Puntius sarana*, *Puntius sophore* and *Crossocheilus diplocheilus*. The family Cichlidae and Bagridae both were represented by only two species namely *Oreochromis aureus*, *Oreochromis massambicus* and *Mystus bleekeri*, *Mystus cavasius* respectively. While the family Mastacembilidae, Channidae, Ambicidae, Heteropneustidae, Siluridae and Sisoridae were comprised of one fish species including *Mastacembelus armatus*, *Channa gachua*, *Chanda nama*, *Heteropneustus fossilis*, *Ompok pabda*, *Glyptothorax cavia* respectively. The study shows that Lower Badrai Stream has good ichthyofaunal diversity but it is still highly influenced by human anthropogenic activities. In this regard, there is need of proper stocking of fishes in Badrai stream.

USE OF DIFFERENT LEVELS OF *MORINGA OLEIFERA* LEAF MEAL IN FISH DIET FOR IMPROVING MINERAL AVAILABILITY IN *LABEO ROHITA* FINGERLINGS

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A 70 - day feeding trial was carried out to study the effect of replacing the dietary fish meal with *Moringa oleifera* leaves meal (MOLM) on the mineral digestibility of *Labeo rohita* fingerlings. Five iso-nitrogenous diets were formulated with MOLM replacing 0%, 10%, 20%, 30% and 40% of fish meal in the diets. Fish were fed at 5% of their body weight two times daily. Fingerlings were randomly distributed into tanks having 15 fish in each replicate. Chromic oxide was incorporated as an indigestible marker in the diets. Results showed that replacement of fish meal with MOLM upto 10% increased mineral digestibility as compared to fish fed on control (0%), 20%, 30% and 40% MOLM based diet, respectively. The present study showed that MOLM has good prospective for use as fish meal replacement in *L. rohita* diet up to 10% level without compromising fish performance. *Moringa oleifera* leaf meal significantly increased mineral

digestibility in *L. rohita* fingerlings at 10% replacement level followed by 20% level as compared to the control diet. On the base of this study it was concluded that fish meal can be replaced with MOLM up to 10% in the diets to increase the nutritive values of *L. rohita* fingerlings.

FISH BIODIVERSITY OF KHIRTHAR HILL TORRENT NAI GAJ JOHI, DISTRICT DADU

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Nai Gaj is one of the largest mountain torrents in south Pakistan, Nai Gaj Nala carries the second highest flow of water after the Indus River and is the main source of fresh and unpolluted water of Manchar Lake. The main stream of Nai Gaj rise from North of Khuzdar and flows generally, southerly direction before turning sharply east, through a deep, narrow gorge in Kirthar Range into the Indus valley. There is a perennial flow of Nai Gaj which covers the area of about 15 Km. This mountain torrent making the large number of depressions (Locally called as KUMBH). The area is famous or the sport fishing. Due to the importance of this huge water body we aimed to clear the fish ecology and biodiversity in Nai Gaj. The sampling was carried out from February to August 2015, however due to flood and other reasons fish samples were not taken during March and July. The sampling was carried out with the various gears (Gill net, Sein net, Mosquito net). Fishing in this area was difficult due to tough geological position and depth of the water. Water temperature was lower in the month of February (14^o C) and higher in the month of August (34^o C; pH ranged between 7.2-7.7). In total 19 fish species in seven families were observed during the study. Maximum species (10) were observed in family Cyprinidae (*Labeo rohita*, *L. dyochelius*, *L. diplostomus*, *Cirrhinus reba*, *Tor macrolepis*, *Tor. Sp.2*, *Salmophasia bascilia*, *Puntius ticto*, *P. sophore*); Three species were observed in family Cobitidae (*Botia almorahae*, *Botia lohachata*, *Botia Kubotai*); family Sisoridae included only *Bagarius bagarius*; family Bagiridae included two species (*Rita rita*, *Mystus bleekeri*), two species in Siluridae (*Wallago attu*, *Ompok pabda*), one species in family Mastacembalidae (*Mastacembalus armatus*); and one species in family Cichilidae (*Oreochromis mossambicus*). The study present the first regular report of Mahasher (*Tor macrolepis*) from Sindh, and another species of Mahsher (*Tor. Sp. 2*) is also recorded. Study also presents the first report of *Botia kubotai* in Pakistan. In future study aims to clear the taxonomy of *Tor. Sp. 2* and seasonal variation in the species composition.

THE NUTRITIONAL IMPACT OF HYBRID CICHLID ON THEIR GROWTH, SIZE, AND LENGTH

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This look at primarily based on "the nutritional impact of hybrid cichlids of their growth, size and length" cichlid fishes are used as the selective breeding "hybrid" applied in the present experiment. Sized, healthy fish fingerlings were procured from the Freshwater decorative aquarium. these fishes use the base of the synthetic weight-reduction plan which chargeable to the growth, size, and length of fingerlings. those experiments based totally on the rearing aquarium where in-person arise and 2nd nursery aquarium both relatively. All the vital fish meal substances Sunflower seeds, oats, wheat, bloodworm, fish rice these are the experimental fish diet which used in the feed of hybrid cichlid. The diet contains fats, vitamins, and another important nutrient which help the fishes' growth, sizes, and length increase. The feeds are mix with essential nutrient herbs as well as the worm which help the great development of fish. After the month located that feed boom the growth, size, and length, and of hybrid fish. The result that displays the feed had a terrific nutritional effect on the growth, size, and length of hybrid cichlid.

FISH BIODIVERSITY OF LOWER LLOYD BARRAGE DISTRICT SUKKUR, SINDH, PAKISTAN

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Studies of fish diversity of lower Lloyd Barrage (Sukkur Barrage), near Sukkur city Sindh were carried out. Monthly fish samples were collected from different locations near Sukkur area. A total of 32 fish species were recorded. These included 22 commercial fish species and 10 non-commercial fish species. The systematic position of ichthyofauna revealed that majority of fish belong to Cyprinidae family, these were 10 in number among which *Cirrhinus mrigala* and *Labeo calbasu* were fairly present the second dominant family was Bagridae in which *Rita rita* was very common, especially when the water was shallow. The third dominant family was Cobitidae in which *Botia lohachata* and its other species were abundant. The murels or snakeheads were next dominant fish group in which three species *Channa punctatus*, *Channa striatus* and *Channa marulius* were present. The present study showed that highly commercial carp fish *Labeo rohita*, and *Catla catla* populations were very low. This indicates over exploitation of these highly commercial fish species. Among the rare species there was *Sisor raddophorus*, belonging to Sisoridae family which was caught once only. The fish diversity is an essential component to understand and manage the aquatic resources. The aquatic resources provide livelihood to thousands of fisherfolk families and food to millions of people. The high demand of fish cause overfishing that results variation in aquatic fauna on large scale. To keep the assets in normal range this study is highly beneficial.

STUDIES ON BIOLOGY OF CATFISH *OMPOK PABDA* FROM INDUS RIVER NEAR JAMSHORO

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Present study was undertaken to elucidate the hematological aspects in relation to Biology of catfish *Ompok pabda* from Indus River near Jamshoro during February to July 2015 through the catch of fisherman from Indus River near Jamshoro. The fish sample comprised of a total 261 specimen (88 male fish and 173 female fish) having total length (TL) 11.2 - 21.5 cm (males) and 10.7- 30.0 cm (females), weighing 9.6 - 48.2 g (males) while females were 6.4- 147 g. The LWRs estimated by linear regression were $\log W = 0.135 + 1.857 \log L$ (for male), $\log W = 0.533 + 0.292 \log L$ (for female) and $\log W = -1.615 + 2.520 \log L$ (for combine population). It was observed that *Ompok pabda* was found to be carnivorous in feeding habit. Three categories of food were discernable as semi digested food consist of (60%) followed by trash fish (25%) and the lowest preference was crustacean (15%) at both length groups. The values of both Gonado-somatic Index (GSI) and ova diameter (egg size) showed continuous rise from February to July with two peaks in March and June. It indicates that fish spawn twice in a year with peak in May.

MARGINAL MARINE FISH PRODUCTION OF PAKISTAN

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It has been observed that in Pakistan total fish production is increasing but marginal fish production is not matching with it. Marginal production mean change in fish output resulting from employing one more unit of particular input i.e. fishing boat/vessels, manpower. The marginal marine fish production has not yet been computed in any study, even government published data base are also absent from this data. In this work last decade marginal marine fish production has been computed. To do a complex model has been developed. That

model has been assigned different singed to different marine production inputs. For instance, increase in numbers of small boat, medium boat and fishing vessel has been give the name of A, B and C inputs. Similarly all model was completed. The notation A, B, C, etc. are compared with the comparative output. Fisheries share in GDP is 0.41%. In the year 2017-17 total marine and inland production was estimated 520,000 m tones, out of which 373,000 is marine product. In the same year these was 15.09%, increase in fish and fish preparation in values and 12.20% increase in quantities term. These authors believe that it is dichotomy of increase, but in real term their poor increase in it. Even inflation values are also not minus from the value of increase. It is a new practice that is not present in calculation of fisheries statistics anywhere in the world.

THE BREEDING BEHAVIOR OF (*CICHLASOMA NIGROFASCIATUM*) CONVICT CICHLIDS

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Cichlids are uncommon among fishes since they have drawn out care of their young. Convict cichlids, *Cichlasoma nigrofasciatum*, are monogamous and have biparental care of their young. This species has been contemplated widely in the lab, in any case, little is known about their reproductive habit in nature. Breeding behavior of convict cichlid was observed from March.2017 to November 2017. Observation indicates that interspecies brood adoption of fry occurs frequently under natural conditions. Pairing was formed by adding all selected fishes. Three aquariums were set with all suitable equipments and water conditions which are needed for these fishes. Temperature, pH and ammonia of water were checked with regular interval. Water temperature was maintained at 27-29°C with aquarium heaters and the photoperiod was 12 h light and 12 h dark. The pH is about 6-7. The glass jars use as a cave for a breeding pairs, because the Convict Cichlids are the cave breeders. In present study it is observed that before breeding the pairs start digging the areas of aquariums by their fins and show aggression. The belly turn into shiny orange color and their black striped on the body turned darker before egg lying. Females breed in the cave and both parents cares their eggs and show aggressive behavior during guarding of their eggs. After the hatching of eggs females protect or care their larvae's or fry's more than males and shows more aggression. The next spawning takes place after 2 weeks interval of egg lying. The fry's were separated from their parents before the second spawning take place because the parents eat their own fry before egg lying of female.

CLIMATE CHANGE AND ENVIRONMENTAL EFFECT ON PRODUCTION OF PALLA, (*TENUALOSA ILISHA*) FROM INDUS RIVER

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The hilsa, *Tenulosa ilisha* (Hamilton 1822), belonging to the family Clupeidae, is locally known as 'ilish' and 'Palla' in Pakistan. Hilsa has a wide range of distribution and occurs in marine, estuarine and riverine environments. It is found in the Arabian Gulf, Red Sea, Arabian Sea, Bay of Bengal, Vietnam Sea and China Sea. The riverine habitat covers the Satil Arab, and the Tigris and Euphrates of Iran and Iraq, the Indus of Pakistan, the rivers of Eastern and Western India, the Irrawaddy of Myanmar, and the Padma, Meghna, Jamuna and other coastal rivers of Bangladesh. Hilsa is largely an anadromous species, capable of withstanding a wide range of salinity and capable of migrating great distances upstream. It migrates to freshwater for spawning. Juveniles develop and grow in freshwater, but soon migrate to sea where they spend most of their life. In the Arabian Gulf, it ascends Shatt al-Arab River in Iraq and other rivers in Iran. Nature of the climate change threat. Fisheries and aquaculture are threatened by changes in temperature and, in freshwater ecosystems, precipitation. Ongoing climate change is predicted to affect individual organisms during all life stages, thereby affecting

populations of Palla species, communities and the functioning of ecosystems. These effects of climate change can be direct, through changing water temperatures and associated phonologies, the lengths and frequency of hypoxia events, through ongoing ocean acidification trends or through shifts in hydrodynamics and in sea level. In general temperature has great influence on change physiology as well as the composition and dynamic coupling of food webs in fish ecosystem.

**MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF PATHOGENIC FUNGUS
ISOLATED FROM SILVER CARP *HYPOPHTHALMICHTHYS MOLITRIX***

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This study was aimed to investigate morphological and molecular characteristics of some fungal species isolated from silver carp. The fishes were obtained from Punjab university research fish farms. The mean total length was 18.32cm and mean body weight was 65.41g (n=162). Infected fishes had eroded scales, damaged caudal and pelvic fins, deep reddish gills and lesions on the body. Fungi isolated from infected parts (gills, skin and fins) of the body of the fishes were cultured on four different media. The inoculated agar plates were incubated for 5–7days at 28–32°C. White, black, grey, brown, and green fungal colonies appeared on agar plates. After isolation fungal colonies were used to characterize the fungi. Polymerase chain reaction (PCR) was done to amplify the ITS region, with ITS1F forward primer and ITS4 reverse primer. Nine genera of pathogenic fungus were morphological and molecular characterized i.e; *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Fusarium equiseti*, *Curvularia lunata*, *Simplicillium obclavatum*, *Penecillium crustosum*, *Neurospora crassa*, *Chaetomium globosum*. The fungal infection observed in silver carp probably occurred through the use of contaminated feed and unsuitable pond environment for fish farming. Fish infection by pathogenic fungi reduces the economic and nutritional value of fish. The present situation points our attention for very good fish health management practices in the fish ponds.

3. MARINE BIOLOGY

DIVERSITY AND DISTRIBUTION OF COMMUNITIES ASSOCIATED WITH MARINE SPONGE *LIOSINA PARADOXA* THIELE, 1899 AT SANDSPIT BACKWATER, KARACHI

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Marine sponge harbors variety of organisms (fauna and flora). A number of communities is being reported from mangrove ecosystem. At Sandspit backwater mangrove (*Avicennia marina*), Karachi coast, sponge samples were collected from four transects during 2013. In the laboratory, samples were washed thoroughly with seawater and immediately retained in polythene bottles (200 mL), preserved in 4% formalin and then transferred in 90% alcohol for community analysis. A total of 931 individuals from 7 major taxonomic assemblages were recorded. Percent composition of fauna represented nematodes (36.6 %; n=341), crustacea (34%; n=317), polychaetes (17%; n=165), foraminifera (9.8%; n= 491), rotifers (0.2%; n=2), helminthes (1.24%; n=114) and lower chordates (0.4%; n=4). Seasonal variation showed highest abundance of crustaceans, nematodes and polychaetes in summer (May, June, and July), respectively whereas foraminifera was abundant in winter (November) season. However, other associated groups (rotifers, lower chordates and larval forms) found in less abundance (0.2-0.6%) throughout the study periods. Physicochemical factors of water were recorded such as, temperature ranged between 19-32 °C, salinity 35-41 PSU, dissolved oxygen 0.11-3.44 mg L⁻¹ and pH 7.04 to 7.69. The results of present study indicated the diversity of associated communities with marine sponge *Liosina paradoxa* is greatly influenced by environmental factors. Furthermore, due to lacking of information, detailed study required to understand the interaction between host-sponge and its inhabitants from coastal waters of Pakistan.

NATURAL DIET OF TWO COMMERCIAL CRAB SPECIES, *PORTUNUS SEGNIS* (FORSKAL, 1775) AND *PORTUNUS SANGUINOLENTUS* (HERBST, 1783), IN THE COASTAL WATERS OF KARACHI

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Crab fishery of Pakistan is supported mostly by four species of family Portunidae. These species are: *Portunus segnis* (formerly reported as *P. pelagicus* from Pakistan), *P. sanguinolentus*, *Scylla serrata* complex and *Charybdis feriatus*. Analysis of stomach contents to discern natural diet is a standard practice in fisheries ecology. It usually aimed to find out if there is any competition for food in the natural environment. *P. segnis* and *P. sanguinolentus* live sympatrically and may or may not compete for food in the coastal waters of Karachi. To find out this, the present study was initiated. The crabs were collected from commercial landings at Korangi Fish Harbour, Karachi. Stomach contents of 558 *P. segnis* (Forsk, 1775) and 426 *P. sanguinolentus* (Herbst, 1783) were examined. The frequency of occurrence and points methods were used for the stomach contents analysis. Mollusca and Crustacea dominated the diet of both the species. Majority of the Mollusca eaten by *P. segnis* and *P. sanguinolentus* were small soft-shelled bivalves followed by small gastropods. Small crabs, isopods, and amphipods were important Crustacea in the diet. Small fish, shrimps, polychaete worms, sponges, starfishes, brittle stars, bryozoans, and plant (algae) materials were also present in some stomachs in small quantity. The two crab species are opportunistic predators and compete on slow moving, small invertebrates. Major reduction in availability of one prey group would hardly effect the crab population.

THE STUDY OF REPRODUCTIVE BIOLOGY OF *SIPHONARIA* ASHGAR FROM THE ROCKY COASTS OF KARACHI

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The specimens of *Siphonaria ashgar* on histological examination were categorized as ambisexual, pure male, predominantly females and predominantly males. Among unisexuals only pure males were found and no pure females were observed. The presence of ambisexuals, predominantly females and predominantly males in the population of *Siphonaria ashgar* throughout the year suggested that the eggs and sperm are always present in the gonads which is in confirmation to other studies which reported that in species of *Siphonaria ashgar* the eggs and sperms were always present within the acini of the gonads (Marcus & Marcus, 1960; Berry, 1977; Simpson, 1977; Hodgson *et al.*, 1991). Pal and Hodgson (2005) reported that *S. capensis* and *S. serrata* were simultaneous and not sequential hermaphrodites. Our study also confirmed the presence of simultaneous hermaphrodites in *Siphonaria ashgar* as the acini contained the male and female gonadal material throughout the year.

THREE AMPHIPODS OF THE FAMILY AMPELISCIDAE (GAMMARIDEA): *AMPELISCA BREVICORNIS*, *AMPELISCA SCABRIPES* AND A NEW SPECIES *BYBLIS INTEGRITELSON* FROM THE PAKISTANI WATERS

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Two genera and three species of amphipod family Ampeliscidae are being described here from the Pakistani waters as two known species of the genus *Ampelisca*—*A. brevicornis* (Costa, 1853) and *A. scabripes* Walker, 1904 and one new species of the genus *Byblis*—*B. integritelson* sp.nov., all of these three are illustrated and described. These two sibling species of *Ampelisca* are compared and their specific differences enumerated. The new species may be distinguished by the combination of certain characters discussed in this paper.

REDESCRIPTION OF THE DEVELOPMENTAL STAGES OF *PILUMNOPEUS CONVEXUS* (MACCAGNO, 1936) (CRUSTACEA: DECAPODA: BRACHYURA: PILUMNIDAE) REARED IN THE LABORATORY CONDITIONS.

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The present paper is based on the result of laboratory rearing of *Pilumnopeus convexus* from the 1st zoeal to megalopa stage. It is the first report of the larvae as those of *Pilumnopeus convexus*. Originally they were described from Pakistan as larvae of the genus *Pilumnus* and compared with the existing larval descriptions of the genus *Pilumnus*. The ovigerous female of *Pilumnopeus convexus* was collected from P.N.S. Himalya, Karachi (Long 66°58'42"E, Lat 24°47'30"N) and maintained in the wet laboratory. Larvae were hatched, at room temperature 18-29°C in filtered seawater of a salinity of 36 ppt and pH 7.9. All the larval stages of *Pilumnopeus convexus* are redescribed supported by with their illustrations, and compared with the available descriptions of *Pilumnopeus* congeners.

COASTAL POLLUTION IN GADANI SHIP BREAKING AREA**Nafisa Shoaib and P.J.A. Siddiqui***Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan*

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This paper discusses marine coastal pollution arising from Gadani ship breaking industry and international conventions on marine pollution. The study analyses existing environmental legislation in Pakistan, its application, and the Pakistan administrative structure overseeing the eco-logical regime. The shipping industry is the backbone of international trade. But these ships have tons of extremely toxic substances, hazardous to human and environmental health. Shipbreaking activities contaminate the environment through the discharge of ammonia, burned oil spillage, floatable grease balls, metal rust (iron) and various other disposable refuse materials. Metals of particular concern that are associated with the ship breaking industry are toxic heavy metals such as lead, mercury and cadmium. There are rich resources in the coastal zone of Pakistan which should be protected from pollution. The fishery industry is an important section of Pakistan's national economy. The quality of fishery products is likely to be impacted by the coastal pollution. Reduction in quantity and quality of fishery products will have a negative effect on the national economy.

ASSESSMENT OF MICRONUCLEI INDUCTION IN SOME COMMERCIALY IMPORTANT FISH SPECIES OCCURRING IN PAKISTAN COAST**Mohammad Tabish, Nafisa Shoaib and Pirzada Jamal Ahmed Siddiqui***Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan.*

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Marine environmental pollution arising from anthropogenic chemicals, such as, heavy metals, pesticides, oil, petroleum hydrocarbons, etc., being drained untreated into the sea from industrial, agricultural, domestic sewage sources. Most contaminants in marine environment are genotoxic and metabolically toxic. In the present study the cytogenotoxicity in wild fish from different areas (polluted and unpolluted) were assessed. Micronucleus (MN) analysis was performed in different fish species caught from both polluted site and relatively clean site. The micronucleus (MN) frequency was recorded from the polluted area suggesting higher level of pollutants in marine environment. In comparison with reference point, relatively low frequencies were recorded. The Micronucleus test gives sensitive result that shows pollution in the coastal area is responsible for the induction of micronucleus in fish and indicates that the MN test in fish is a suitable biomarker for in situ monitoring of genotoxic pollution in the marine environment.

DISTRIBUTION AND ABUNDANCE OF MARINE TURTLES IN COASTAL AND OFFSHORE WATERS OF PAKISTAN**Muhammad Moazzam***WWF-Pakistan, 46-K, PECHS, Block 6, Karachi 75400, Pakistan*

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Five species of marine turtles are known from Pakistan. Of these, green turtles (*Chelonia mydas*) which are most common turtle species found in coastal waters, nest on a number of sandy beaches during October and February along Pakistan coast. Hawksbay, Taq (Ormara), Astola Island and Daran (Jiwani) are the main nesting area along Sindh and Balochistan coast. The nesting population is observed to be decreasing during past three decades but still a large population of green turtle is known from offshore waters. Olive Ridely turtle (*Lepidochelys olivacea*) used to nest along Pakistan coast but for last 13 years no nest was located along Pakistan coast, although a very large population has been observed in the offshore waters. Three other species of marine turtles i.e. loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*) and leatherback

((*Dermochelys coriacea*) turtles are represented by only a few specimens from Pakistan coast, however, there is no authentic record of their nesting in Pakistani waters. Hawksbill turtle is considered to be critically endangered species according to IUCN Red List and green turtle is regarded as endangered whereas olive Ridely, loggerhead and leatherback are considered to be vulnerable. Data collected from WWF-Pakistan's observer programme which was started in 2012 revealed that in the offshore waters olive Ridely turtle is the most dominating species with their peak abundance season during September and December and February and May. Green turtles were found throughout the year with peak during October and December and during April. Although marine turtles are facing a number of threats which is affecting their population in Pakistani waters but their interaction with the fishing gears is possibly the most important factor which results in mortality. It is estimated that annually about 29,000 turtles used to get entangled in offshore gillnet fisheries, however, recent initiative taken by WWF-Pakistan of introduction of subsurface gillnets has resulted in major decrease in entanglement of marine turtles. During 2016 only 2,700 were recorded to be entangled in offshore gillnet fisheries. The mortality which was found to be about 2.5% in 2013 and 2014 was observed to have been reduced to only 0.04%. The paper also discusses management implication of gear modification on marine turtle population in Pakistan.

**POPULATION STRUCTURE AND TEMPORAL VARIATION IN THE GONADAL DEVELOPMENT
IN THE POPULATION OF *MERETRIX CASTA* (FAMILY: VENEROIDAE)
FROM SONMIANI, IN PAKISTAN**

Shahnaz Jahangir, Ghazala Siddiqui, Zarrien Ayub, Qamar, N., Panhwar, S.K and Noreen Farooq
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The sequential dissimilarity, sex-ratios, size-frequency in gonadal development and spawning season, of *M. casta* var. *ovum* from Sonmiani were studied. The overall sex-ratio in *M. casta* var. *ovum* was slightly in favor of females. In eight months of the sampling period sex-ratio was close to expected 1:1 theoretical ratio. In October, December 2004, February and April 2005 significant departure from the expected 1:1 ratio was observed in favor of females. The females dominated in October 2004, February and April 2005. Males were more in number November and December 2004. No indifferent clam was found in this species. In the size-classes >30 and <80 mm the sex-ratios were significantly close to 1:1. The gonadal development in males and females takes place throughout the year without any resting period. The high frequency of developing females in the population was recorded in the months of July and August 2004. The ripe females were encountered in eight months of the sampling period. The major peak was recorded in January 2005. The spawned out females were more frequent during September to November 2004 period. The ripe males were found in six months of the sampling period. Their major peak was recorded in August 2004. The resorbing females appeared in four months of the sampling period. Their highest percentage was observed in July 2004 followed by March and the lowest was found in May. Resorbing males were present throughout the study period except in June, December 2004 and in April 2005. In males the peak was recorded in January and their lowest number was found in July 2004. The spawning season in females was observed throughout the year except, August 2004 and April 2005. The peak of spawning in females was recorded in October 2004. The spawning in males was noted in ten months of the year except, November and December 2004. The spawning peak of spawning in males was observed in August 2004. The peak of GI was recorded in September and October-04, while its lowest value was recorded in December-04.

**SURVIVAL AND RESISTANCE POTENTIAL OF *CERITHIDEOPSIS CINGULATA* (GMELIN,
1791) AGAINST COPPER METAL UNDER DIFFERENT ECOLOGICAL CONDITIONS**

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Cerithideopsis cingulata has been studied for biological remediation along the Karachi coast in

mangrove ecosystem which is found to be heavily loaded with industrial and agricultural effluents. Regardless of these serious ecological threats, this species found abundantly in mangrove swamps. Keeping in view its remarkable potential, Static bioassay method was used to determine acute toxicity of copper using 0.25ppm, 0.5ppm, 1.00ppm, 1.5ppm and 2.5 ppm concentrations along with different ecological conditions i.e. solar radiation, pH (alkaline and basic), salinity (20‰ and 40‰) and temperature (10°C, 20°C, and 30°C) at 96h exposure period. In acute toxicity experiment LC₅₀ was observed at 96 hr. under controlled laboratory conditions. At different ecological conditions, resistance of *C.cerithideopsilla* was found to be varied accordingly i.e. at 20°C, 48 hr. LC₅₀ was observed. It is inferred from the study that *C.cerithideopsilla* resist the maximum concentrations up to the maximum time, showing its tendency against selected metal and the best survival was found at solar radiation proceeding 30°C and alkaline pH.

ESTIMATING GROWTH AND MORPHOMETRIC CHARACTERISTICS OF TWO EDIBLE CRABS OF GENUS *PORTUNUS*, FAMILY PORTUNIDEA BY USING LENGTH-WEIGHT RELATIONSHIPS

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The study deals with the relative growth and morphometric characteristics of two species of edible crabs, namely *Portunus pelagicus* and *P. sanguinolentus*. Early studies available on crabs were belongs to the relative growth of brachyuran crabs on benthic and burrowing species while, recent study includes swimming crabs belonging to the family Portunidea. A very little work on *Portunus* crabs, on relative growth and size-weight relationship from Pakistan has been published so far. About 54 specimens of different sizes were collected from the Manora Beach and Karachi fish harbour, during February to December 2017. Out of which, 34 specimens were belonging to *P. Pelagicus* and 20 specimens were belonging to *P. sanguinolentus*. Total length of *P. Pelagicus* was ranging from 9.5cm to 13.9cm while, in *P. sanguinolentus* was 9.2 to 15.2 cm. The relationship of length-weight was found negatively correlated in both species. Size-weight relationship showed that males were heavier than females in both species. According to the seasonal measurement results show the high growth rate of crabs during winter season in both species.

TAXONOMIC ASSESSMENT OF THE FAMILY FICIDAE MEEK, 1864 (1840) (GASTROPODA: MESOGASTROPODA) WITH A NEW RECORD AND DISTRIBUTION ALONG THE PAKISTAN COAST (NORTHERN ARABIAN SEA)

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Molluscs are considered one of the most diverse groups of animals and they are widely distributed throughout the world. The largest class are the Gastropoda and accounts for 80% of the total known living species. The family Ficidae is commonly known as “fig shells”, which are mainly found in tropic and warm temperate seas. This family consists of two genera: *Ficus* Röding, 1798 with eleven known species and three subspecies, and *Thalassocydon* (Barnard, 1960) having three accepted species at present. Species of *Ficus* are frequently found in a sand, silt and/or mud habitat. Furthermore, Ficidae have a rather complex historical nomenclature with numerous synonyms. Ficidae Meek, 1864 (1840) is the currently accepted taxon on a family level. None of the species have been recorded from Pakistan earlier, though two species have been reported previously, namely *Ficus gracilis* (G. B. Sowerby I, 1825) and *Ficus variegata* Röding, 1798. In the present study, we report three species collected from the intertidal zone during detailed beach surveys along the Sindh (89 Km) and Balochistan (96 Km) coasts. Specimens were identified based on their morphological characters.

A total of three species belonging to the genus *Ficus* - including two previously reported species and one new record: *Ficus ficus* (Linnaeus, 1758) - are being reported from Pakistan. All species are here described with detailed taxonomic and morphometric features. A new distribution record and an identification key to the family ficiidae are included.

MORPHOMETRY AND MORPHOLOGY BASED TAXONOMY OF FISH IN THE FAMILY MUGILIDAE (JAROCKI, 1822) FROM PAKISTAN COAST

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Family Mugilidae (Jarocki, 1822) is generally considered to be ecologically important and is widely distributed in coastal waters of tropical and sub-tropical zones. Fish belongs to family Mugilidae are commonly known as "mullets". This family has 18 genera and 82 species. The taxonomy of family Mugilidae is ambiguous because of lack of disparity among the species of this family. Paucity of literature makes it necessary to study fish taxonomy and to develop identification keys. The objective of proposed study is to identify the species of Mugilidae family based on morphological parameters. For this study work fish samples were procured from different localities of Sindh and Baluchistan coasts. Detailed morphometric and meristic characters of each specimen such as total length, standard length, measurement of different body parts with proportion to standard length, presence or absence of pectoral axillary scale, number of longitudinal and transverse scales and soft rays of 2nd dorsal, anal and pectoral fins were studied for the identification of these specimens. In the view of recent and past literature all the important taxonomical details of species of Mugilidae family have been studied thoroughly. With the help of this data we were able to study new characteristics of this unique family by giving colored photographs and proper identification key. The present paper describes important taxonomic key characters along with otolith characters of tunas species of Indian seas. The present paper describes important taxonomic key characters along with otolith characters of tunas species of Indian seas.

IMPACT OF DIFFERENT SALINITY LEVELS ON GROWING PERFORMANCE, FOOD CONVERSION AND MEAT QUALITY OF RED TILAPIA (*Oreochromis* SP.) REARED IN SEAWATER TANKS

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The effect of varying salinity levels from 15‰ to 40‰ with 5‰ increment on growth, feed efficiency, meat quality and survival rate of Red tilapia, *Oreochromis* sp. (mean body weight 5±0.07g) were investigated. Juveniles of red tilapia were randomly distributed into seawater tanks (60 × 30 × 45 cm each). Ten fish were stocked in each with 2 replicates for each treatment. Fish were fed with commercial floating pellet (35% protein) at 3% body weight per day for 40 days. Results show that fish growth was found to be significantly ($P<0.05$) higher in term of weight gain, WG % of initial weight, mean daily WG, SGR, feed conversion and survival rate from 15‰ to 30‰ salinity than those reared on 35‰ and 40‰ salinity. Condition factor found significantly higher on 40‰ than 15‰ to 35‰. Feed conversion ratio was non-significantly different in all salinity levels. Biochemical analysis of fish meat showed that moisture, protein, lipid, ash and crude fiber were not significantly ($P>0.05$) affected by salinity level. The hematological parameters like hematocrit, cholesterol and plasma triglycerides were similar among fish fed on different salinity level ($P<0.05$). Present study proves that red tilapia can be reared up to 30 ‰ salinity to get optimum growth and maximum survival rate.

ESTIMATING GROWTH AND MORPHOMETRIC CHARACTERISTICS OF TWO EDIBLE CRABS OF GENUS *PORTUNUS*, FAMILY PORTUNIDEA BY USING LENGTH-WEIGHT RELATIONSHIPS

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The study deals with the relative growth and morphometric characteristics of two species of edible crabs, namely *Portunus pelagicus* and *P. sanguinolentus*. Early studies available on crabs were belongs to the relative growth of brachyuran crabs on benthic and burrowing species while, recent study includes swimming crabs belonging to the family Portunidea. A very little work on Portunus crabs, on relative growth and size-weight relationship from Pakistan has been published so far. About 54 specimens of different sizes were collected from the Manora Beach and Karachi fish harbour, during February to December 2017. Out of which, 34 specimens were belonging to *P. Pelagicus* and 20 specimens were belonging to *P. sanguinolentus*. Total length of *P. Pelagicus* was ranging from 9.5cm to 13.9cm while, in *P. sanguinolentus* was 9.2 to 15.2 cm. The relationship of length-weight was found negatively correlated in both species. Size-weight relationship showed that males were heavier than females in both species. According to the seasonal measurement results show the high growth rate of crabs during winter season in both species.

NEW RECORD OF SHOVEL-NOSED LOBSTER *THENUS UNIMACULATUS* BURTON AND DAVIE, 2007 (CRUSTACEA: DECAPODA: SCYLLARIDAE) FROM THE COASTAL WATERS OF PAKISTAN

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The confirmation of new record of shovel-nosed lobster genus *Thenus* Leach, 1815: *T. unimaculatus* Burton and Davie, 2007 from the coastal waters of Pakistan. Twenty six specimens of *Thenus unimaculatus* collected from the Karachi fish harbor Pakistan. The confirmation of this species based on morphological, morphometric and molecular (mitochondrial coding gene Cytochrome oxidase COI) approaches. *Thenus unimaculatus* has been evaluated as data deficient in the IUCN Red List of threatened species: Red list Category and Criteria: Data Deficient version 3.1, (IUCN, 2011). *T. unimaculatus* previously only identified from Thailand, United Arab Emirates, and Mozambique and due to inadequate data their exact distribution pattern is remain indefinite. In addition, the studies dare lacking on habitat, distribution abundance, population, and intimidations to *T. unimaculatus*. Additional studies are prerequisite for the further precise conservation assessments.

SHELLS SELECTION PREFERENCE BY HERMIT CRAB *CLIBANARIUS PADAVENSIS* (DIOGENIDEA: ANOMURA: DECAPODA) FOUND IN INTERTIDAL MANGROVE AREAS OF PAKISTAN

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Hermit crabs (Diogenidea: Anomura: Decapoda) are the most morphologically, ecologically diversified and shell associated fauna present in the intertidal zones of Pakistan coast. The aim of this study was based on the preference of gastropod shells by a *Clibanarius padavensis*; frequently distributed species on the shores of Sindh and Baluchistan. The preference of shells was computed with percent association to discriminate species of gastropod based on occupied shells by the species. The different Morphometric relationships were estimated

between shells and crabs as per their availability and size preference of gastropod shells. Specimens were collected from different mangrove and Halophyte associated muddy areas during August 2016 to December 2017 from 6 sites (Korangi Creek, Russian beach, Ketu Bunder, Sonari beach, Bhanbhore creek and Sonmiani bay). The 25 species of gastropod shells were recorded from which the two gastropod species *Telescopium telescopium* (36.6%) and *Thais carinifera* (30.11%) showed the highest preference however; the 12 shell species were found only approximately only 1% preference.

**ANALYSIS OF PHYLOGENETIC RELATIONSHIP OF MACROPHTHALMID CRAB
WITH GRAPSOID AND OCYPODOID CRABS BASED ON
MITROCHONDRIAL DNA (COI) MARKER**

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Crabs of genus *Macrophthalmus* play an important role as detritivore feeders and burrow constructor in intertidal as well as subtidal marine coastal habitats. They are the most typical inhabitant of sandy muddy substrate along IWP region, having great morphological diversity. In *Previous studies*, the *Macrophthalmid* crabs were placed together with *Grapsid* and *Ocypodid* in the Catometopa but in the recent classification of Brachyura these crabs placed separately with fiddler and sand bubble crabs within superfamily Ocypodoidea (subsection thoracotremata) whereas *grapsid* placed separately within superfamily Grapsoidea. Present study is conducted to elucidate the phylogenetic position of crabs of genus *Macrophthalmus* from Pakistan based on mtDNA (COI) marker. The results based on neighbour joining (NJ) and maximum parsimony (MP) methods; showed close phylogenetic affinities of the *Macrophthalmid* with *Grapsoid* crab than the other crabs of same super family including *Dotillid* and fiddler crabs.

**SPECIES RE-DESCRIPTION OF GENUS OCYPODE (FAMILY: OCYPODIDAE) THROUGH
BIOCHEMICAL AND GENETIC ANALYSIS ALONG THE COAST OF PAKISTAN**

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Ghost crabs are commonly found along the coastal belt of Pakistan. The review of literature revealed that no previous research has been done based on biochemical and molecular work except few preliminary taxonomic studies on genus *Ocypode*. Biochemical analysis carried out by using five isozyme markers Carbonate Dehydratase (CD), Catalase (CAT), Amylase (AMY), Peroxidase (PXD) and Octanol (Oct) and two general proteins by using different stain (Coomassie brilliant blue and Amido black). Among these markers CD found as a distinguishing marker to discriminate the species with significant difference ($P=0.00$) which deviate the HW-equilibrium. Higher level of genetic differentiation ($F_{ST}=53.3\%$) found between two species indicating species are quite distinct from each. On molecular basis, combined data set shows that the three species (including one new species) form two highly supportive monophyletic clades with 100 bootstrap values when applied through ML, MP and BI. Genetic distance also showed higher level of variation between three species (5.5%). *Ocypode rotundata* showed higher level of intra-specific variation in isozyme (37% polymorphic loci) and molecular data (genetic distance = 0.7%). The three species have been confirmed by the use of four mitochondrial DNA Markers and three nuclear DNA markers. The ongoing further analysis for crabs of different localities and usage of different molecular markers includes (ITS-1 and ITS-2) likely resolve and discriminate the further more species of this genus.

INVESTIGATING THE NUTRITION AND GROWTH PERFORMANCE OF POST FRY TO FINGERLING STAGE OF BLACK FIN SEA BREAM UNDER DIFFERENT CONTROL CONDITIONS

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The objective of the present study was to investigate the growth, feed consumption and body composition of black fin sea bream (*Acanthopagrus berda*) reared on two different feeds i.e. T₁-floating feed and T₂- Sinking feed in glass tanks (60 liters) holding capacity for 40 days. Seed of experimental fish were collected from Sonari chenal (sindh coast) and then procured into the aquaculture research laboratory of the CEMB. After completion of acclimatization period seed were distributed among treatments having mean initial length and weight (5.84±0.2g, 4.47±0.2 cm) respectively. Both feeds were supplied 2 % of total biomass two time per day at 9:00 A.M, and 4:00 P.M. At the end all fishes were collected and measured in total length (cm) and weight (g). Results shows that the highest weight was found on floating feed (6.226±0.41g), while the lowest weight was observed on sinking feed (6.008±0.17g). Feed conversion ratio (FCR) were not significantly different among treatments (P<0.01). Survival remained 100% in both treatments. Condition factor was found to be significantly different among the groups (P<0.01). Specific growth rate (SGR) were significantly different among treatments (P<0.05). Water quality parameters (temperature 28.02±0.13°C, dissolved oxygen 7.31±0.32mg/l, pH 7.59±0.14, ammonia 0.067±0.004mg/l) were recorded throughout the study period and found within the tolerable levels. It is suggested that the culture of Blackfin seabream, *Acanthopagrus berda* on floating feed on similar condition will be more profitable.

NEW RECORD OF (MOLLUSCA: BIVALVIA: CHAMIDAE) CHAMA GRYPHOIDES LINNAEUS 1758 and SPONDYLUS LIMBATUS LINNAEUS 1758 FROM ROCKY SHORE BULEJI ALONG THE COAST OF PAKISTAN (NORTHERN ARABIAN SEA)

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Bivalves are the diversified organisms of fresh and marine water habitat. They are ecologically important due to the faster metabolic mechanism and filter feeding capability. In this context, the family Chamidae and Spondylidae belong to the class Bivalvia. It has been evaluated that the Chama species and Spondylus species are in abundance at rocky shore, Buleji: Balochistan. However, the new data is limited regarding Chamidae and Spondylidae family. The transposition of Chama shell into left and right valve attachment creates an ambiguity between two Genera that Chama and Pseudochama. Also, the Chamids are termed as Jewel-Boxes due to high variability in colouration and morphology of the shell and have remarkable similarity with the family Spondylidae. However, the Spondylus species contains large shell with a wing similar to Pectinada. The Chama and Spondylus species have been assessed by the conchological studies referring to literature. The *Chama gryphoides* Linnaeus 1758 and *Spondylus limbatus* Linnaeus 1758 are recorded for the first time from the Buleji Pakistan. The aim of the study is to identify the members of Chamidae and Spondylidae Family and assess the taxonomic hierarchy in perspective of evolution for first time in Pakistan. This study also concludes that the new record of Chamidae and Spondylidae provides an addition to the assets of Marine Bivalve Fauna of Pakistan. It is recommended that further study is required in regard of commercially important chamids and spondylids.

COMMUNITY STRUCTURE OF MACROBENTHIC INVERTEBRATES IN MANGROVE SWAMPS OF KORANGI CREEK, KARACHI (NORTHERN ARABIAN SEA)

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Korangi is known as one of the polluted coast of Karachi due to the presence of domestic and industrial pollutants. Korangi creek is dominated by the presence of the mangrove, *Avicennia marina* serving as a nursery ground for many marine organisms. Macrobenthic invertebrates thus play an important role in assessment of the environment condition in understanding the ecosystem of an area. Macrobenthic invertebrates in Korangi creek is mainly composed of Crustaceans, polychaetes, gastropods, bivalves, echinoderms and Cnidarians. A variety of gastropods are however present as the most dominant invertebrate in Korangi mainly consisting of *Architectonica* sps, *Bullia*, *Cerithium* sps, *Littorina* sps, *Natica didyma*, *Polinices*, *Turbo canaliculatus* and *Rhissoria* sps. Other invertebrates were also found but relatively in less numbers. In Bivalves, *Donax*, *Mercenaria* and *Lithophaga* are the main benthic invertebrates. Crustaceans mainly represented by mangrove crabs such as *Uca* and *Macrophthalmus* in fairly good numbers. Polychaetes are relatively in less numbers composed of *Neries* & *Diopatra* sps. Echinoderm is only represented by a Sea cucumber, *Holothuria*. Cnidarians were the most least abundant macrobenthic invertebrate occurring in only few months and is represented by Sea anemones. Physical parameters are also included in the study. Seasonal variation of macrobenthic invertebrates among the transects and the inter tidal fauna is also discussed.

DECADAL VARIABILITY OF PRIMARY PRODUCTIVITY OF ARABIAN SEA INFLUENCED BY GLOBAL ANNUAL MODIFICATION OF SUN SPOT

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Our world is facing the problem of food due to climatic shifts. Due to climate change the variation occurs in Marine ecosystem where Primary productivity plays an important role in the oceans, they are basic food for Marine fishes and other aquatic organisms. The emphasis of this study is to understand the relationship of Global Modification in sunspot with Primary productivity in Arabian Sea. The investigation was done by comparing annual Chlorophyll Concentration (Chl. Conc.) and annual variation in Sunspot from the years 2002 to 2014. The Grid Analysis and Display System tool is used for manipulation that revealed the spatial and temporal variability of regional MODIS Aqua Chl. Conc. and its distribution over Arabian Sea. Further analysis is carried out by comparing Chl. Conc. with Sunspot. This investigation shows weak correlation and data is statistically significant.

SEASONAL VARIABILITY IN SHELLFISH DIVERSITY AROUND KARACHI NNP

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The data from thirteen hauls from demersal trawls operated around Karachi NPP were used to evaluate shellfish diversity and dynamics in 2016-17. Forty-nine species belongs to seven groups (Shrimps, Crabs, Cephalopods, Lobsters and Molluscs) were recorded in overall catches during the entire sampling programme. In all, Gastropods encountered with highest (24) number of species, followed by crabs (8) and Loligo (cephalopod) comprises of single species with highest biomass 52 kg. Twenty-five species of the Molluscs belonging to the class Gastropoda and Bivalvia were also collected. Moreover, pooled data of species

occurrence in four respective seasons defines that highest (30 spp.) were caught in September and the lowest (17 spp.) in March, whereas in May and December 20, 23 species respectively. To determine effects of habitat characteristics (temperature, salinity, pH, depth, and dissolved oxygen) on the shellfish communities (49 cases) was tested with Canonical Correspondence Analysis (CCA).

A CHECKLIST OF MARINE LIFE PREDATORS ALONG THE KARACHI COAST

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The marine life is widely divided into tapped and untapped fisheries. Destruction of tapped fisheries remained under objection by early writers. On the other hand, untapped fisheries are totally ignored. To enlist the predators, this study classified Marine life into five segments. Namely, Commercial fishes, Commercial Crabs, Ornamental fishes, Ornamental invertebrates and Shell fisheries. In this study it is noticed that some variables of destruction are common in all of the above five fisheries, i.e: Over harvesting, Environmental Pollution, Urbanization, Industrialization, etc but it has been observed that some variables of destructions of Marine life are not yet been reported. For instance, (a) Children of coastal villages killing dozens of coastal wildlife as a fun, (b) The development of Marine Aquarium fisheries is washing out shore fisheries, i.e baby Green Sea turtle is having whole sale price of PK Rs.100/- per piece. Zointhod fauna is 25% destructed by them aquarium fish collector. Moreover, Karachi Coast is having six world top most beautiful Zointhod. These Zointhods are attached with rocks and are unable to escape from the collectors. Anyhow, in this study a list of wildlife predator has been compiled for above noted five categories of marine life. In this list the predator of tapped and untapped fisheries is mentioned separately. This study found twenty five major predators of marine life, in which non target catcher and fishes collected for poultry feeds are on top index of destruction of wild life.

MARKETING, BIODIVERSITY, CHECKLIST, REARING AND THREAT OF POLYPS OF KARACHI COAST

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Coastal belt of Karachi is 135 km long and about 13% of country's coast. Numerous species of polyps are widely distributed along all rocky tidal zones. In Pakistan, Polyps are a tapped resource for selling to marine fish aquarium shops. It is found that Pakistan has 4 species from the world top 10 zaonthids (Polyps). Our Bam bam, Tulips etc. are really having dark color and greatly attracting the local marine fish hobbyist. In turn, local collectors are eradicating some of these zaonthids from rocky shore i.e. "Kaka Pir" Hawksbay. It is reported that for rearing the Polyps, moderate light and flow of water is the executive key. Conservation through commercialization is become necessary at Karachi coast. The prevailing whole-sale price for a 5 square inch polyp is Rs.100 in Karachi. The local fisherman is very comfortable in collecting polyps with hammer and chisel. Collecting and selling polyps are far easier than any other fisheries. Another, outcome of the study is that coastal fauna and flora of Karachi coast is tremendously increasing for the last couple of years. Consequently, it positively impact biodiversity as new species are growing at every sea site.

POOR USING OF STING RAY FISH BEING FOOD & MEDICINAL REASON IN PAKISTAN**Mohammad Idrees², Hafiz Syed Firdaus¹, Nadeem Ahmed Tamimi³ and Yasir Ali Siddiqui³**¹*Department of Neurology, Jinnah Postgraduate Medical Centre, Karachi*²*Dr. Idrees Clinic, Haroon Heights, E-14, 11-K, North Karachi*³*Pakistan Air Force, Karachi Institute of Economics and Technology, Karachi*

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About ten species of sting ray are thickly distributed along Sindh coast. Its commercial and medical uses are very poor in Pakistan. Due to so its whole sale prevailing prices are Rs.150/= per KG. It is very low comparatively. It is observed that the Pakistan population is not taking other zinc rich sea food, due religious prohibition doubt. This prohibition doubt is also encircle the sting ray and sharks. Therefore, in this study prohibition doubt is removed by taking that Fatwah from the top most religious university/Madrassa of Sindh. Local source reported that taking of this fish, can reduce the intensity of Parkinson. The authors are reporting the self-case study for reduction of Pakistani intensity in them by use the cheetah species of sting ray. The Papri and Gadami species of sting ray also commonly harvested as commercial edible species into the recent year. The zinc deficiency of Pakistan population is also reported and clinically observed by the authors. It was observed in three hundred patients that their genital power is increased tremendously after one time consumption of this fish. In female patient it is observed that taking of this fish is increasing the hardness of breast and made them well standing. Our survey reveals that in common food item local population are taking zinc rich food once, in three months.

4. PALAEOLOGY

NEW ARTIODACTYL FOSSILS FROM DHOK PATHAN FORMATION OF PAKISTAN

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The Late Miocene-Early Pliocene of Pakistan has produced a rich artiodactyl fauna. The new fossils include the cranial material of artiodactyls. The diverse material presents taxonomy of artiodactyl from the Dhok Pathan Formation of Pakistan. The assemblages of artiodactyls from the Potwar Plateau are dominated by the presence of the bovids. The taxa are consistent with a Late Miocene-Early Pliocene age of the deposits. The specimens are classified on the basis of morphometric features of the material from the Late Miocene-Early Pliocene of the Siwalik Group. This faunal list may be compared with that of other Late Miocene localities of the Siwaliks. The artiodactyl remains increasingly indicate both taxonomic and adaptive diversity.

CORMOHIPPARION (EQUIDAE, MAMMALIA) FROM MIDDLE SIWALIKS OF PUNJAB, PAKISTAN

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The *Cormohipparion* horses are abundant in North America and relatively less abundant in Europe and Africa. *Cormohipparion* is present in the Middle Siwalik Subgroup of Pakistan as well but, here, its occurrence is very rare. Its lower premolars and molars can be easily identified by the presence of protostylid, which is intact with protoconid. The protoconid is longer rather than round as in *Sivalhippus*. We describe isolated dentitions of hipparionine from the Middle Siwalik Subgroup (11.2–3.4 Ma) of the Potwar Plateau of Northern Pakistan, and assign the material to the species *Cormohipparion* sp.

STEGOLOPHODON (PROBOSCIDEA, MAMMALIA) FROM SIWALIK GROUP, PUNJAB, PAKISTAN

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Fossil proboscidea have been eye catching to palaeontologists and were, indeed, the earliest vertebrate fossils to be studied and described. Proboscidea is represented by five families in the Siwaliks and one of these is Stegodontidae, represented by two genera *Stegolophodon* and modern form *Stegodon*. The genera are almost restricted in the Siwaliks. *Stegolohodon* is represented by three species. *Stegolophodon stegodontoides* ranges from Lower Siwaliks to Middle Siwaliks. New remains of *Stegolophodon stegodontoides* recovered from the Siwaliks of Pakistan include upper and lower premolars and molars. Here we describe dentitions of recently excavated from the Potwar Plateau area of Pakistan, which provides evidence that, indeed, the proboscidean species has existed in Pakistan.

**MERYCOPOTAMUS DISSIMILIS (ANTHRACOTHERIIDAE, MAMMALIA)
FROM MIDDLE SIWALIKS OF PUNJAB, PAKISTAN**

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Anthracotheriids are extinct artiodactyls, present throughout the world including the Siwaliks. Their record goes back to pre-Siwaliks but these are abundant in the Siwaliks too, especially were very diversified in the Middle Siwaliks, which includes the Nagri and Dhok Pathan formations. *Merycopotamus dissimilis* is one of the dominant species of the genus *Merycopotamus*. This genus is represented by three species in the Siwaliks. The genus *Merycopotamus* is only known in Asia and is inhabitants of aquatic environments because of their frequent presence in lignite and lacustrine deposits. New remains have been reported from the Siwaliks, including dentition, jaw fragments and a partial skull. The paleogeographic distribution of *Merycopotamus dissimilis* is discussed. The localities, which yielded anthracotheriid remains, are briefly discussed.

**NEW REMAINS OF *PROGIRAFFA EXIGUA* FROM KAMLIAL AND CHINJI
FORMATIONS OF LOWER SIWALIKS, PAKISTAN**

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New remains of *Progiraffa exigua* are recorded from the Kamlial (ca. 18.3–14.2 Ma) and Chinji formations (ca. 14.2–13.2 Ma) of the Lower Siwaliks. The new findings of *Progiraffa exigua* extend its stratigraphical range from the Kamlial Formation to the Chinji Formation of the Lower Siwaliks. Prior to this study, the species was only restricted to the Early Miocene of the Subcontinent. The material is collected from Jaba, Rakh Wasnal, and Ghungrila. The material comprises mandible and maxillary fragments, and isolated upper and lower dentitions. The Siwalik *Progiraffa* is congeneric with *Prolibytherium* or *Injanatherium* of Eurasia. Giraffidae made their first appearance in the Kamlial Formation of the Siwalik Group.

**NEW REMAINS OF MIDDLE MIOCENE *GAZELLA* (BOVIDAE, RUMINANTIA)
FROM DHOK BUN AMIR KHATOON, PUNJAB, PAKISTAN**

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New remain of *Gazella* from Dhok Bun Amir Khatoon of Lower Siwalik Subgroup are described. The recovered material comprises of thirteen specimens including maxillary and mandibular fragments, and upper and lower isolated teeth of varying preservation states. The metric and morphological features like narrow styles and external folds, no entostyle in the upper molars, the lower molars with goat folds and a small ectostylid support their inclusion in *Gazella* sp. This study adds information on anatomical morphology of Middle Miocene *Gazella* sp.

**NEW REMAINS OF THREE TOED HORSES FROM THE DHOK PATHAN LATE
MIOCENE, PUNJAB, PAKISTAN**

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This research project describes new fossils of three-toed horses with detailed systematic study originating from the Dhok Pathan Formation of northern Pakistan. The studied material comprises nine specimens including upper and lower isolated dentitions. These tetracuspid, narrow crowned teeth with isolated protocones clearly showed that specimens under study belonging to two genera namely *Hipparion* and *Sivalhippus*. Careful morphometric measurements and comparison of these specimens led to the documentation of three species: *Hipparion* sp., *Hipparion antilopinum*, *Sivalhippus* cf. *theobaldi*. The sample adds new information on the anatomical morphology of Late Miocene horses of the Siwaliks.

**NEW REMAINS OF *EQUUS SIVALENSIS* FROM THE PABBI HILLS
(UPPER SIWALIKS) OF PAKISTAN**

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The studied samples collected from the Pabbi Hills of Pakistan are comprised of dental remains of an extinct horse, *Equus sivalensis*. The genus *Equus* had its origin in North America, and it subsequently migrated to Eurasia. The genus *Equus* in Asia is known by three species, namely *E. sivalensis*, *E. namadicus* and *E. Palaeonius*. The first species was discovered from the Pleistocene Siwalik deposits of both Pakistan and India, while the latter two species are only reported from the Pleistocene of Narmada Valley (India). The *Equus sivalensis* was specialized by the protocone that is not isolated in the upper premolars and molars in contrast with the primitive genus *Hipparion* in which the protocone is isolated. In *Equus sivalensis* the teeth are highly hypsodont. The lower molars are relatively small sized and narrow having thin enamel with shallow ectoflexid and metastylid and have V-shape lingual depression with well-developed plicaballoid that is more directed at posterior side in *Equus sivalensis* as compared to other species of *Equus*.

**DESCRIPTION OF *BISON* FROM THE UPPER SIWALIKS
OF PUNJAB, PAKISTAN**

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The Siwaliks bovids are diverse with variations in their body size and have great taxonomic stability in Pakistan. However the large sized bovid fauna is reported in the Upper Siwalik strata. The dental remains of *Bison* cf. *sivalensis* described in this paper were collected from the Tatrot and Pinjor strata exposed near the Tatrot village in district Chakwal and the Pabbi hills in district Jhelum respectively. The *Bison* along with the associated mammalian fauna of the Upper Siwaliks including; *Leptobos*, *Proamphibos*, *Rhinoceros*, *Tetralophodon*, *Equus* and *Anancus*, inhabited the open wood land area and swampy habitats. The fossil remains of *Bison* are also known from the Late Pliocene and Pleistocene strata of Eurasia, America and Africa.

**GIRAFFOKERYX PUNJABIENSIS (MAMMALIA, ARTIODACTYLA) FOSSILS FROM
THE CHINJI FORMATION OF NORTHERN PAKISTAN**

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New fossil remains of family Giraffidae have been described and discussed in this study. The discovery includes isolated upper and lower dentition belongs to *Giraffokeryx punjabiensis*. The identification is based on comparative morphometry with already published data. This comparative study shows that *Giraffokeryx punjabiensis* is a brachyodont and smaller in size species having middle Miocene geological range in the Siwaliks of Pakistan. The present study strengthens the existing knowledge about the geography, stratigraphy and paleoclimate of this extinct giraffid species.

**ENAMEL HYPOPLASIA ANALYSIS IN DEINOTHERES (A FAMILY OF EXTINCT ELEPHANTS)
TO TRACE OUT THE MIOCENE STRESS EVENTS IN THE SIWALIKS OF PAKISTAN**

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The Deinotheridae is an extinct family of proboscideans (elephants) known from the Siwaliks of Pakistan. The occurrence of this taxon at Middle Miocene epoch in different regions of the world indicates its migration because of stressful environmental changes. The impact of the Middle Miocene stress events on extinct elephant species is traced out in this study by enamel hypoplasia analysis. Enamel hypoplasia is a mammalian dental defect that can be a reliable stress indicator. The study includes 34 dental samples belong to two species *Deinotherium pentapotamiae* and *Deinotherium indicum* that were analyzed by two observers at different light intensities. *D. pentapotamiae* showed 17%, whereas, *D. indicum* presented 25% enamel hypoplasia. The difference in occurrence of enamel hypoplasia in these two species is statistically non-significant that inferred a high level of stress for overall Middle Miocene Siwalik proboscideans fauna of the Siwaliks. The studied molars and premolars have 27% and 12% hypoplasia respectively, this shows that the stress episodes were linked with the less availability of food and continued loss of natural habitats for the animals because of changing environmental conditions. Stable warm temperature of the Middle Miocene might be a significant contributor for these Miocene stress events that pushed the Siwalik Deinotheres towards extinction within a very shorter life span at the end of middle Miocene relative to the European *D. giganteum* and the African *D. bozasi*.

**TRACING OUT THE SIWALIK PLIOCENE STRESS EVENTS BY ENAMEL
HYPOPLASIA ANALYSIS IN CERVIDS**

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Enamel hypoplasia is a tooth malady resulted as depletion of enamel forming cells called ameloblast. It is a reliable and permanent marker for the stress events in the life history of mammals. Five Siwalik extinct species named as *Cervus Sivalensis*, *Cervus triplidens*, *Rucervus simplicidens* and *Axis punjabiensis* are

included in current cervids enamel hypoplasia analysis. The 33.33% cervids of early Pliocene time interval (Dhok Pathan Formation) has occurrence of EH while for early to late Pliocene epoch (Tatrot Formation) this percentage is 45%. The statistical results point out a significant increase in occurrence of EH in the Siwalik cervids from early towards late Pliocene ($p < 0.05$). This incremental increase of stress for cervids during Pliocene epoch is related to drier, cooler and seasonal environment of the Pliocene interval in the Siwaliks of Pakistan. Open lands of Pliocene age also add up to the predatory stress for cervids.

**NEW COLLECTION OF FOSSIL REMAINS OF *CONOHYUS INDICUS* FROM
THE SIWALIK HILLS OF PAKISTAN**

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New fossil remains of *Conohyus indicus* have been studied from Middle Miocene to Late Miocene rocks of Lower Siwaliks and Middle Siwaliks of Pakistan. The genus *Conohyus* contains relatively small and medium sized tetraconodonts having enlarged third and fourth premolars. It is oldest known genus of subfamily Tetraconodontinae. In Siwaliks the genus *Conohyus* is known by two known species, *C. indicus* and *C. sindiensis*. It entered the Indian subcontinent in Kamlial and ranges upto Nagri formations. It is poorly known tetraconodont from the apex of the Lower Siwaliks and base of the Middle Siwaliks. The collected material consists of fragmentary mandibular and maxillary parts as well as isolated teeth.

5. WILDLIFE, DIVERSITY AND CONSERVATION

DISTRIBUTION AND DIET OF COMMON LEOPARD (*PANTHERA PARDUS*) IN DISTRICT SUDHANOTI, AZAD JAMMU & KASHMIR, PAKISTAN

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The common leopard (*Panthera pardus*) is "Critically Endangered" in Pakistan while IUCN Red list categorizes it globally as "Vulnerable". The current study investigated its distribution and diet composition in District Sudhanoti of AJ&K. Distribution of the species was determined using "Sign Survey" method while its diet composition was investigated by "Scat Analysis". During surveys, a total of 28 scats, 14 pug marks, 8 prey remains and 7 dead bodies of common leopard were recovered in an approximately 262 km² scanned area. The scats were mostly found on tracks and trails in hilly terrain, pug marks were recorded in riparian zone bank of river and nallas, while its prey remains were found mostly in dense bushes and rocks. The dead bodies of common leopard were recovered within or around the village areas. All signs of the species were found between 418 m to 2016 m elevational range above sea level (asl). Scat analysis revealed a total of 10 prey species of common leopard including seven domestic mammals and three wild meso- mammals. Domestic animals were most frequently consumed by common leopard in the study area (73.66% F) while wild prey contributed 26.31 %F. The domestic prey species included goat, sheep, dog, cow, buffalo, donkey, and horse, while three wild prey species were Rhesus monkey (*Macaca mulata*), Red fox (*Vulpes vulpes*) and hare (*Lepus capensis*). The consumption of domestic prey species was higher in summer (88.21%) season, while wild prey were consumed more heavily (38.08%) in winter season. The Chi-Square test showed significant difference (0.001) in consumption of domestic prey by common leopard in the study area, whereas consumption of wild prey did not differ significantly (0.90).

DISTRIBUTION AND POPULATION OF BLACK BEAR (*Ursus thibetanus*) IN DISTRICT MANSEHRA, PAKISTAN

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Two species of bear are reported from Pakistan viz; Asiatic black bear (*Ursus thibetanus*) and Himalayan brown bear (*Ursus arctos*), having 3 well-known sub-species including Baluchistan black bear (*Ursus thibetanus gedrosianus*), Asiatic black bear (*Ursus thibetanus laniger*) and Himalayan brown bear (*Ursus arctos isalbellinus*). The black bear usually inhabits deciduous forests, thorn brush forests, and diverse forests, hardly occurring on or above 3,700 m above sea level. The current study aimed at studying distribution, and population of Asiatic black bear in District Mansehra during 2016-2017. For recording distribution of the species, direct and indirect signs of black bear were recorded. A transect methodology for signs survey and questionnaire was used to estimate the population of the species. A total of 66 indirect signs of Asiatic black bear were recorded in the study area with an altitudinal variation of habitat ranging from 1511m to 2570m elevation in Kaghan valley, and 1830m to 2447m elevation in Siran valley. Overall distribution range of Asiatic black bear was found between 1511m to 2570m elevation in the study area. A total of 17 dens of the species were found in the area while 44 scats of the species were recorded from 10 different sampling sites. The sign density of black bear was highest at Paris MRF (17/km²), followed by Paris Jabra (15/km²) and least at Sharan (1.2/km²) site. Maximum sign density was found between elevation of 2240m - 2360m. The sign density was high in habitat having thick, dense and broad-leaved forest with steep slopes consisting of water resource.

**DISTRIBUTION, POPULATION AND FOOD HABITS OF GREY WOLF (*CANIS LUPUS*)
IN SULEMAN RANGE, SOUTH WAZIRISTAN, PAKISTAN**

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Grey wolf (*Canis lupus*), one of the largest species of Canid family, occurs in various parts of the country including South Waziristan. It is categorized as "Endangered" in the country and reportedly, its population is decreasing in its range due to various factors. Data regarding various ecological aspects of the species are scanty in the country, therefore, the current study investigated distribution, population, and food habits and human-grey wolf conflict in the Suleman range, South Waziristan. The distribution of grey wolf in the study was determined by conducting field surveys and recording its direct and indirect signs like scats, pug marks, foot prints, whereas its population was assessed by estimating its sign density such as occurrence of dens, scats, and pug marks while its food habits were studied by analysis of its scat samples. The grey wolf was recorded at eight out of 12 sampling sites surveyed with an elevational range between 1642 m to 2688 m asl. A total of 6 dens, 60 pug marks and 41 scats of grey wolf were recorded in the study area. Maximum predicted individuals of grey wolf were at sampling site 2 "Larkhoo" (10 individuals). Thus, a careful estimate showed that total predicted population of grey wolf at selected sites was approximately 41 individuals, with a density of 1.7 individuals / km² area surveyed. Scat analysis revealed 4 domestic and six wild prey species in the diet menu of the grey wolf, with 52% contribution from livestock and 48% from wild prey. The domestic prey included goat, sheep, cow and donkey while wild prey species in its diet menu were Rhesus monkey, Cape hare, Royale's Pika, house mouse, Red fox, and wild boar. The study concludes that the Suleman Range, South Waziristan is an important area regarding distribution of grey wolf, however, existing low population and its retaliating killing demands conservation measures to be launched in the study area to save its remaining population.

**DIVERSITY, DISTRIBUTION AND ABUNDANCE OF EARTHWORMS IN DISTRICT
NEELUM, AZAD JAMMU, AND KASHMIR, PAKISTAN**

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In current research, ecological diversity and species richness of earthworms were examined in the different localities of district Neelum, Division Muzaffarabad, Azad Jammu and Kashmir, Pakistan. The collection was conducted during August, 2017 to September, 2017. Earthworm collection was made in forest soil, Municipal solid waste dumped area and paddy field. Earthworms were collected by hand sorting and digging procedures. Population density, Shannon diversity index and encounter rates for each species were calculated. *Aporrectodea caliginosa*, *Aporrectodea longa*, *Pheretima lignicola*, *Allolobophora chlorotica*, *Pheretima posthuma*, *Aporrectodea trapezoides*, *Aporrectodea rosae* were identified morphologically. These identified earthworms belong to Epigeic and Endogiec species. It was observed that various localities especially high elevated areas decline the earthworm diversity due to snow and cold temperature. Highest earthworm abundance was recorded in various villages of Kern and Athmuqam while minimum in case of Kel, Arang kel up to Taobut localities. The *Aporrectodea* species were dominant in all localities. The population density and percentage was also recorded. The whole observation clearly indicated that the earthworm's richness was very low in the district Neelum.

MORPHOTAXONOMIC STUDY OF VARIOUS GENERA OF ORDER CHIROPTERA**Afshan Ashfaq Ahmed***Department of Zoology, University of Sindh, Jamshoro.*

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The study of morphotaxonomic of various genera of order chiroptera inhabiting district Hyderabad, conducted from 17th march 2017 to 15th august 2017, which is consisting the group of 1st sub order "Megachiroptera" of family "Pteropodidae" included genus "Pteropus" species "Pteropus giganteus" and 2nd sub order "Microchiroptera" of family "Emballonidae, Vespertilionidae" fauna of different 25 specimens including number of 15 males and 10 females of genus "Taphozous, myotis" of species "Taphozous perforatus, Myotis lucifugus, Yuma myotis" were studied and identified with international parameters with the help of key of Texas and book the mammals of Pakistan by T.J Robert. The captured species measured with various parameters as under. (i) Body length (ii) Body weight (iii) Length of tail (iv) Length of fore limbs or wings (v) Length of hind limbs or foot (vi) Length of ears (vii) Length of tail. These parameters have measured with different variation of body, color, nose and ears.

STATUS, DISTRIBUTION AND THREATS TO SCALY ANTEATER (*MANIS CRASSICAUDA*) IN SOUTHERN BELT OF KHYBER PAKHTUNKHWA, PAKISTAN**Mohib Ullah Khan and Zia Ur Rahman Awan****Department of Zoology, Govt. Post-Graduate College Bannu, Khyber Pakhtunkhwa Pakistan*

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Pangolins are placental mammals also known as Scaly Anteater are included under the order Pholidota with eight existing species in the world. The body of pangolin remains covered with hard keratinized scales which provide it distinct and unique characteristics from others. The Indian pangolin is an endangered species often inhabit specific habitats and are dependent on specific prey. The main prey items of Indian pangolin include two species of black ants and one species of termites. The present study was conducted with aim to know the status, distribution and threats to scaly anteater (Pangolin) in southern districts (Bannu, Lakki and Dera Ismail Khan) of Khyber Pakhtunkhwa, Pakistan. The animal was found in both plains and hilly regions of the study areas. Pangolin lives in burrows and each burrow is used by a single individual. Population density showed a sharp and significant decreasing. All populations have declined and are now perceived to be very low abundance. Only 14 respondents consider pangolins to remain locally present and these respondents all regard the status of pangolins as rare. At present habitat destruction has drastically decreased the wild population of pangolins, pushing them to the edge of extinction. Captive breeding is an important way to protect this species. The recorded population decline indicated that conservation measures may be needed.

MORPHOLOGICAL STUDY OF VARIOUS VARIETIES OF ASEEL CHICKEN BREED INHABITING DISTRICT HYDERABAD**Madiha Qureshi***Department of Zoology, University of Sindh, Jamshoro*

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Aseel is the most popular and historic breed of chicken with many varieties found all over the world. The study was designed to explore the morphological variation of Aseel chicken varieties in district Hyderabad. A survey was conducted during 5th April 2017 to 23rd August 2017 in four localities of district Hyderabad including Tandojam, Goth karan khan shoro, Tower market and Railway line colony. A total number of 54 samples (20 males and 34 females) of six varieties of Aseel chicken breed (Sindhi, Mottled, Black, Lakha, Jawa, kulang) were studied and identify with different morphological characters such as comb type, size of wattles and earlobes, plumage color, shank color, beak color and eye color. The collected varieties of Aseel breed were measured with different body parameters as under: (i) Body length from tip of beak to cloaca (ii)

Body weight (iii) Comb length (iv) wattle length (v) Beak length (vi) length of tail from cloaca to tip of tail (vii) length of fore limb (viii) length of hind limb. These parameters have measured with different morphological variability in different varieties with variation in plumage color and patterns, size and comb type. Great morphological diversity were observed among these varieties and this study provide a baseline information for future research in the area.

PRELIMINARY ASSESSMENT OF DIVERSITY AND DISTRIBUTION OF EARTHWORMS IN LOCALITIES OF DISTRICT MUZAFFARABAD, AZAD JAMMU, AND KASHMIR, PAKISTAN

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Earthworms are the most important components of soil biota in terms of soil formation and maintenance of soil structure and fertility. In current research, diversity of earthworm species was examined in different localities of Muzaffarabad, Azad Jammu and Kashmir, Pakistan which are not studied before in this area. A total of 18 earthworm species such as *Eisenia fetida*, *Aporrectodea caliginosa*, *Aporrectodea longa*, *Pheretima lignicola*, *Amyntus minimus*, *Amyntus gracilis*, *Amyntus hupeiensis*, *Polypheretima elongate*, *Lumbricus terrestris*, *Perionyx excavates*, *Allolobophora chlorotica*, *Pheretima posthuma*, *Aporrectodea trapezoides*, *Aporrectodea rosae*, *Drawida nepalensis*, *Pheretima hawayana*, *Bimastos parvus* and *Eisenia andrei*, respectively were morphologically identified from Muzaffarabad. The diversity and distribution of earthworm species related to the species abundance was also investigated. It was observed that the distribution of earthworms in different regions is influenced by different soil factors like temperature, pH, moisture contents, and soil texture. The purpose of present study is to collect and identify the earthworm species from different localities of Azad Jammu and Kashmir which might be helpful to elaborate the importance of soil formation and soil fertility due to existence of earthworms in the field of agriculture and might be helpful for further studies and research on earthworms. It was concluded that *Aporrectodea trapezoides*, *Amyntus minimus*, and *Aporrectodea rosae* found in rich organic soil and would be suitable to enhance the production of vermicompost according to geographic condition.

STUDY OF POPULATION DYNAMICS AND DIVERSITY OF SNAILS IN VARIOUS AGROECOSYSTEMS OF MULTAN, PAKISTAN

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Snails are second largest group of invertebrates and make a major part of the world fauna next to insect. They provide intermediate host for parasite and predators. In the molluscs, the gastropods are only class which have successfully occupy on land. There are 35,000 described species of land snails (subclass: Pulmonata) from class gastropods in the world. Many species of land snails harm various crops and act as notorious pests. With the present scenario of climate change, their occurrence has been observed many-fold. Despite their economic importance, the studies on population diversity and distribution of these creatures in Punjab, Pakistan on different host crops are not much more. The present study aims to identify the snails' species diversity in agroecosystems of Multan. The snails were collected from water channels and different crops like sugarcane, mango orchards, fodder and vegetables from the District Multan. A total of 2,000 specimens were collected and

exposed to sun light, and then treated with hot water to remove their soft parts. Then they were washed with fresh water and preserved dry shell and also in 70% alcohol and stored in Ecology Lab, MNS - University of Agriculture, Multan. The preserved specimens were studied under microscope with the help of taxonomic keys. *Oxyloma elegans*, *Oxychilus draparnaudi* and *Ariophanta* spp. were identified so for the most prominent were *Oxyloma* spp. Relation of irrigation water quality like ground, canal and sewerage water with population dynamics of snails was also studied. The result revealed that the maximum population recorded from the crops irrigated by sewerage and canal water. The population diversity was at maximum in sugarcane and mango orchards than fodder and vegetables fields. The maximum population was found in July and August. The abrupt change in population was recorded after rain and population were maximum in rainy season then the winter season.

DIVERSITY AND DISTRIBUTION OF ORNITHOFAUNA IN RURAL AREA OF TEHSIL PATTOKI

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The present study was designed to observe avian diversity and abundance in rural areas of Tehsil Pattoki. A total of 117 species were observed in five selecting sites during survey period. Among these 38 year round resident, 18 were winter breeder, 25 were summer breeder, 4 were year round visitor, 7 were winter visitor, 9 were passage migrant, 5 were irregular year round visitor, and 1 was a common winter visitor. Field surveys were carried out to record the bird diversity from August 2016 to February 2017 and the study sites were visited during dawn and dusk. Bird species were identified with the help of binoculars and field guide (Grimmett et al., 2008). The diversity index indicated a high level of biodiversity with Shannon wiener index of 4.49 in rural areas of Pattoki. The species richness at site 1 was 78 and total 1379 birds were observed. The most abundant species recorded during the survey period were Common myna, Bank Myna, Cattle Egret, Little Egret, White breasted Kingfisher with a Shannon wiener index of 4.44. At site 2, 56 species were observed with a diversity index of 4.36. Likewise the species richness at site 3 was 100 and a diversity index of 4.49. a total of 90 species were identified at site 4 ($H' = 4.40$) while at site 5 68 species were present with a Shannon wiener index of 4.43. The most abundant species at all sites were House sparrow (RA = 6.12%), House crow (RA = 12.10%) and Common Myna (RA = 10.45%). It was concluded that despite increasing urbanization and expanding industries, the area is still rich in ornithofauna although generalist feeding species are becoming more abundant.

WINTERING WATERFOWL DIVERSITY AT RAWAL LAKE, ISLAMABAD

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Every year during winters, large number of birds from Central Asia and Europe migrate towards Pakistan via Indus Flyway. The birds reach Pakistan flying over Karakorum, Suleiman and Hindu Kush Ranges along the Indus River. Falcons, cranes, swans, ducks, flamingos, waders and geese are important migratory birds in Pakistan. The high global significance of Pakistan's wetlands is attributable to the diversity of species they support. Wetlands in Pakistan provide feeding, nesting and breeding grounds to migratory avifauna. Situated in humid sub-tropical zone in the Southeast of Islamabad, Rawal Lake is an important wetland of Margallah Hills National Park. Rawal dam is constructed on Kurang River and has a catchment area of 106 square miles. Lake stores 84,000 acre feet of water in an average rainfall year (1000 mm). Four major streams and 43 small streams contributing to its storage. The reservoir serves as important water supply source for the cities of Rawalpindi and Islamabad. Lake is also a recreational point for the dwellers of twin cities. Every year a significant proportion of migratory birds inhabit the lake as their wintering ground. Present study was carried out from November 2016 to March 2017 and data on diversity of waterfowls at Rawal lake Islamabad was

collected during time hours of day between, morning (6.00 am to 10.00 am) and late afternoon (4.30 pm to 7.00 pm). Three fixed survey points were selected around the lake and birds were observed using spotting scopes and binoculars and identified using the field guides. GPS points of the survey sites were recorded and periodic boat surveys were also carried out in order to gain a better picture of bird diversity. The data was analyzed using Microsoft Excel 2010 to determine species richness and relative abundance. Shannon-Wiener diversity index (H) was also employed to explore the diversity. A total of 40 bird species from 9 families were recorded during five months of lake survey. These families included *Anatidae* (64 %), *Phalacrocoracidae* (11%), *Ardeidae* (10.5 %), *Laridae* (8%), *Charadriidae* (2.16 %), *Scolopacidae* (1.59 %), *Rallidae* (1.57 %), *Podicipedidae* (0.97 %) and *Recurvirostridae* (0.24 %). According to Shannon Diversity Index, Lake hold a moderate bird diversity i.e. $H' = 2.6588$. Maximum number of birds was observed in February 2017 i.e. 1,682 individuals, while minimum number was recorded in December 2016 (91 birds). In migratory waterfowls, Northern Shoveller was most abundant species ($n = 736$, $R.A = 0.1992$, $H' = -0.3213$) and Common Pochard ($n = 2$, $R.A = 0.0005$, $H' = -0.0038$) with least abundant species recorded. In local migrant, Black headed gull was recorded with highest numbers ($n = 298$, $R.A = 0.0806$, $H' = -0.2029$) while, two birds species viz. crested great grebe and common red shank ($n = 1$, $R.A = 0.0002$, $H' = -0.0017$) were lowest in numbers. As the lake is a recreational area, it is necessary to protect the niche of migratory birds. Lake pollution also needs to be monitored along with construction activities near the buffer zone to protect the visiting avifauna.

POPULATION ABUNDANCE, DISTRIBUTION AND THREATS TO SCALY ANTEATER (*MANIS CRASSICAUDATA*) IN POTOHAR REGION OF PUNJAB, PAKISTAN

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Indian pangolins (*Manis crassicaudata*) commonly known as scaly ant eater is a mammalian species with rigid keratinized scales around its body. They are nocturnal, burrowing and eat ants and termites. Pangolins play important ecological roles, especially their diet of ants and termites make them natural pest controllers. They are widely distributed in Indian sub-continent from Pakistan throughout much of the India (South of Himalayas), Bangladesh, Sri Lanka, possibly Myanmar and extreme West of China. Information regarding population status and other biological and ecological aspects of pangolins is scarce due to its nocturnal behavior. The species is under tremendous hunting pressure due to high demand of its scales in illegal wildlife market. Much of this trade is routed to China for traditional Chinese medicines. Reports of mass killings of Indian Pangolin were highlighted in the media making it a priority concern for conservation in Pakistan. Previously conducted studies on the species have warned about the local extinction if poaching is not controlled. Keeping in view the tremendous hunting pressure and ecological importance of the species, attempts were made to study the population status of pangolin in the selected sites of Potohar region i.e. District Chakwal, Jhelum and area of Fateh Jang in District Attock and Margalla Hills National Park. Extensive field surveys of the open wild areas i.e. potential habitat site for Scaly ant-eater were carried out during October 2014 to July 2015. GPS points of study sites were recorded and details of the burrows were quantified and photographed. Powerful electric chargeable Torches were used in the field area during night surveys and at least four to five hours were spent in field. Information regarding the illegal capture of species in study region was collected via interviews with the local people belonging to different backgrounds including shop keepers, farmers, nomads, business men and students. A total of 26 fresh living burrows, 68 old burrows, 47 feeding burrows and 2 direct sightings of species were recorded from 11 selected sites of District Chakwal. There is a record of 8 fresh living burrows, 31 old burrows and 24 feeding burrows from District Jhelum with maximum population density in the area of Sohawa. In District Attock a total of 14 km² area was surveyed. 15 fresh living burrows, 32 old burrows and 27 feeding burrows were observed in the six studied sites. Maximum population density of Indian Pangolin in Potohar Region was recorded in Chumbi Surla Wildlife Sanctuary i.e. 0.008/hectare. As compared to Potohar Region, a sufficient population of Indian Pangolin was observed in Margallah Hills National Park (0.016/hectare). A total of 11 fresh burrows, 27 old burrows and 15 feeding burrows were recorded from an estimated area of 7 km of the Park. Despite the direct and indirect records of the species during the field visit, information regarding illegal hunting and trade of the species revealed that the animal is highly prized for its scales and is under tremendous hunting pressure and much of its population has vanished. If present trend of

declining population continues, it is no doubt that the species will face high risk of extinction in near future. There is a dire need to address the conservation issues of Indian Pangolin. Concrete measures need to be taken to control the illegal trade of the species on the part of Federal and Provincial Wildlife Departments along with imparting education and awareness to the masses for the conservation of biological capital.

A TRANS-BOUNDARY STUDY OF PUBLIC TOLERANCE OF SNOW LEOPARD AND WOLF IN THE PAMIR

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The escalating human-carnivore conflict which is essentially economically fueled, hinders the coexistence and survival of snow leopard and wolf in the remote mountain areas such as the Pamir. Appreciation of the underlying factors affecting public tolerance of carnivores is prerequisite to formulate informed conservation management measures. This study attempts to assess the intensity of livestock predation by snow leopards and wolves and public perceptions generating thereof, across the Afghanistan, Pakistan, and Tajikistan Pamir Mountain range through questionnaire surveys. It also relates other major causes of livestock mortality in the region with predation induced losses. We used multivariate logistic regression to test that which socioeconomic factors influence and best describe human attitude towards large carnivores in R. Overall, 1,419 (315 per year and 1.7 per household per year) livestock were reportedly killed by snow leopards (47%) and wolves (53%) in the three study sites as compared to 2,868 animals (637 per year and 3.5 per household per year) that died due to diseases. The average economic loss due to diseases was estimated to be higher (US\$ 352 per household per year) as compared to predation (US\$ 191 per household per year) across the study sites. People with comparatively smaller landholdings and limited earning options, other than livestock rearing, expressed negative attitudes towards both wolves and snow leopards and vice versa. Similarly, educated people showed more tolerance towards predators as compared to illiterate. Low public tolerance of the wolf and snow leopard in general explains the magnitude of the human induced threat to predators in the Pamirs. It is highly suggestive to initiate tangible conservation measures like establishing trans-boundary protected areas and community based conservation programs such as disease control, corral improvement and livestock schemes across the Pamir mountain range to safeguard these endangered species with the march of time.

ASSESSMENT OF AVIAN FAUNA OF KALAR KAHAR LAKE DISTRICT CHAKWAL, PAKISTAN

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The aim of the study was to evaluate the avian diversity especially of aquatic avian fauna at Kalar Kahar Lake from October 2015 to September 2016 on monthly basis. During the survey total 40 species were observed and recorded at the Kalar Kahar Lake with the total count of 7581. Common Coot (*Fulica atra*) was recorded as the dominant species with the total count of 3667 while Little Egret (*Egretta garzetta*) was recorded as sub dominant species with the total count of 542. Least counted species was Eurasian Marsh Harrier (*Circus aeruginosus*) with a total count of 3. Out of total 40 species which 55% were Resident, 35% were winter visitors, 7% were summer breeder and 3% were passage migrant. Census index of the lake was calculated

which was 5678.7/Km². Shannon-Weiner diversity index was 2.23. Evenness index was 0.6 and richness index was 4.3. Relative abundance of each species was calculated.

A STUDY ON DISTRIBUTION AND DIVERSITY OF AVIAN FAUNA AT LUDEWALA BYPASS, SARGODHA

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The study was conducted to evaluate the diversity and distribution of avian fauna at Ludewala bypass Sargodha. Study started from October 2015 to September 2016. The study includes avian fauna diversity and distribution according to seasonal changes. Total number of observed avian species at Ludewala was 66 and total numbers of birds observed during this study were 7167. Shannon-Weiner diversity index was maximum (3.81) for the month of March and minimum (3.47) for the month of July. Evenness was maximum (0.94) for the months of March and minimum (0.89) for the month of July. Richness index was maximum (21.42) for the month of February and minimum (17.19) for the month of May. The study area contains thick vegetation, crops and water which facilitate the productivity of living organisms.

COMPARATIVE STUDY ON EXTERNAL MORPHOMETRIC PARAMETERS OF EGGS OF VARIOUS AVIAN SPECIES

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Eggs are economically very important. Eggs are not only source of food but it also uses in pharmaceutical, food processing and cosmetic industries. The aim of the study was to explore the morphometric parameters of various avian species in granting scientific information for their proficient usage in industries and research purposes. The present study evaluated morphometric parameters of eggs among the five avian species namely the Domestic Geese (*Anser anser domesticus*), Pea Fowl (*Pavo cristatus*), Turkey (*Meleagris gallopavo*), Guinea Fowl (*Numididae*) and Partridge (*Perdix perdix*). The significant higher egg weight was observed of domestic geese (157.10±3.01g) followed by Pea fowl, Turkey, Guinea fowl and Partridge. Significant higher egg length (6.85±0.06), egg breadth (4.82±0.07), egg volume (79.27±2.79) and egg surface area (88.45±2.09) was observed of Domestic geese followed by Turkey, Pea fowl, Guinea fowl and Partridge. Significant higher egg shape index of Pea fowl (77.30±0.34) followed by Partridge, Guinea fowl, Domestic geese and Turkey. Higher egg shape index shows the eggs are rounded. Egg surface area, Volume and shape index are geometrical properties and use to predict the chick condition. Comparison of all these parameters shows that there is a significant difference among the external quality parameters of different avian species eggs.

THE ANAEROBIC CO-DIGESTION OF POULTRY DROPPINGS AND COW MANURE WITH SEWAGE SLUDGE: AN ENVIRONMENTAL REMEDY

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Anaerobic co-digestion is a striking tonic to the problem of waste disposal. Mixing of different waste enhance the methane production by improving the digester stability due to their interaction effects. In this study

an efficient strategy is described that involve adjusting best ratio of sewage sludge, cow manure and poultry droppings by using interaction effects to improve methane generation. The experimental results indicate that the maximum methane generation was 197.18 cm³/400g/day with 76% methane content by mixing sewage sludge, cow manure and poultry droppings in 1:1:1 ratio at mesophilic temperature range in a batch reactor. SPC 2 shows highest VFA s 813 /g/mL. Bioslurry of this mixture was proved to be a good soil conditioner against DAP.

COMPARISON OF SEASONAL VARIATIONS AND ABUNDANCE OF BIRD SPECIES IN RURAL AND URBAN AREAS OF DISTRICT SARGODHA

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The changes in habitat structure and resources are due to urban development and birds responds to these changes easily. Identification of avian diversity was conducted through point method and by using the direct (observations) method with binoculars (10x50) and spotting scopes of different magnifications. Data was collected at dawn and dusk times (usually from 6:00-9:00am and from 4:00- 6:00pm). Our results from the study of bird diversity clearly differentiate the urban bird community from rural community. Rural areas had more number of bird species than urban areas. Bird community is less in urban areas due to urbanization, habitat destruction, pollutions, human activities and land use habitat.

ASSESSMENT OF AVIFAUNA OF RANGPUR LAKE, DISTRICT KHUSHAB, PUNJAB

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A survey was conducted at Rangpur Lake from October 2015 to September 2016 on monthly basis to assess the avian diversity. During this survey a total of 110 species and 39146 birds were recorded. Shannon-Weiner diversity index 3.8, Margalef index 10.3 and Evenness index 0.81 were calculated. Dominant species was Little Egret (*Egretta garzetta*) with the total number of 3823 birds and sub dominant species was Indian Cormorant (*Phalacrocorax fuscicollis*) with the total number of 2852 birds. Sarus Crane was least observed with total count of 4.

EFFECT OF DIFFERENT DIETARY PROTEINS ON BEHAVIOR OF PEKIN DUCK (*ANAS PLATYRHYNCHOS DOMESTICA*) IN CAPTIVITY

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To study the effects of different dietary protein concentration on behavioral characteristics of Peckin duck (*Anas platyrhynchos domestica*) thirty day-one old female ducks were selected. They were fed *ad libitum* along with different experimental diets. Experimental diets were the varying levels of protein concentrations in diet. The three levels of protein concentrations are 16.5%, 21% and 22%. Ducks were randomly distributed into three groups each comprising ten ducks and received protein treatment for four months. Behavior was observed on daily basis. Following behavioral activities were noted i.e. aggression, preening, feather pecking, walking, sitting, standing, ground pecking, drinking, snapping water, bill washing, bathing, feeding, body shaking, head shaking, tail shaking, head scratching, bill scratching, voice call, wing flapping, beak fence, threat and rubbing.

Significantly ($p < 0.01$) high preening, walking, tail shaking, body shaking and head shaking behaviors were observed in high protein concentration (22%) treatment. While aggression, feather pecking and sitting behaviors were significantly higher ($p < 0.01$) in low protein concentration (16.5%) treatment. This study concludes that increased dietary protein concentration has positive influence on behavior shown by Peckin duck.

STUDY ON THE SEASONAL VARIATIONS IN DIVERSITY OF AVIFAUNA IN CHASHMA LAKE, MIANWALI, PAKISTAN

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The seasonal variations in the diversity of avifauna in Chashma Lake were examined during this one year study from April, 2016 to March, 2017. Chashma reservoir was divided into three sub lakes which were Main Lake, Haji's Lake and Dera Lake. Data were collected by direct and indirect methods. Binocular and spotting scope was used to enumerate the birds. A field Guide to birds of Pakistan by Z.B Mirza was used as a key for the identification of birds. Total 30740 birds were counted belonging to 23 species, 17 families and 11 orders. Shannon-Wiener diversity index was minimum during December (1.43) while maximum during May (2.72).

FIRST RECORD OF *CLINOSTOMUM MARGINATUM* IN BLACK-CROWNED NIGHT HERON *NYCTICORAX NYCTICORAX* FORM HAMAL LAKE SINDH PAKISTAN

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A study was conducted on Black-Crowned Night Heron, *Nycticorax nycticorax* of Hamal Lake from October 2016 to September 2017. Twenty four hosts were examined in Parasitology laboratory of Zoology department Sindh University. The content of visceral organs was examined on stereodissecting microscope. Result revealed that seven hosts were infected with 134 specimens of genus *Clinostomum* Leidy, 1856. The careful and technical observation uncovered that specimens belong to *Clinostomum marginatum* Wright, 1879. However, this species being reported for the first time in Pakistan.

FEEDING AND BREEDING ECOLOGY OF ASHY-WREN WARBLER (*PRINIA SOCIALIS*) IN POTHWAR PLATEAU, PAKISTAN

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Ashy-wren warbler (*Prinia socialis*) is a common inhabitant of the agro-ecosystem of Pothwar plateau, Pakistan but data on its feeding and breeding ecology in this area are lacking. We investigated the diet and breeding of this bird in farmlands of Pothwar plateau. The analysis of 200 fecal samples showed that *P. socialis* is an insectivorous bird and feeds on flies, mosquitoes, bugs, termites, aphids, weevils and beetles. Breeding data revealed that these birds nest in low shrubs during May to September. We found 30 nests having 112 eggs in total. Mean clutch size was $3.73 \pm 0.79SD$ while average incubation period and nestling period was 11.65 ± 0.52 days and 11.89 ± 0.68 days respectively. Hatching, fledging and breeding success was 62.06%, 50% and 31.03% respectively. The main threats to the species were predation, human disturbance and harsh weather.

POLLUTION ASSESSMENT OF ROAD TUNNELS IN CHINA AND PAKISTAN**Romaisa Qureshi* and Zulfiqar Ali***Environmental Health and Wildlife, Department of Zoology, University of the Punjab, Lahore*

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Air is an ambient mixture of different gases with fractions and water vapours. An air pollutant could be in the form of a solid particle, liquid droplets or as gases. The objective of this research was to assess air pollution in 44 road tunnels of China i.e., Chengdu to Lang Musi Zhen (32°39'35N, 103°36'09E at 2876m elevation) and 5 tunnels of Pakistan i.e., Shishkat to Attabad (36°39.747N, 074°50.586E at 2839m elevation). Metrological parameters, CO₂ concentration along with different particulate matter were assessed using standard protocol. Independent t-test showed PM₁, PM_{2.5}, PM₁₀ and PM_{total} concentrations for China and Pakistan were non-significantly different from each other with p-value 0.071, 0.069, 0.071 and 0.07 respectively while temperature, humidity and carbon dioxide were significantly different from each other having p-value 0.000, 0.000 and 0.011 respectively which are also justified by Pearson co-relation. Spearman's correlation assessed relationship between different parameters and their associative behaviour and showed temperature was non-significantly correlated with particulate matter, humidity and CO₂. While PM_{2.5}, humidity and CO₂ were significantly correlated. CO₂ concentration as linear with vehicular emission was higher inside of tunnel in china but traffic blockage in Pakistan rise its high levels outside of it. The confinement of tunnel's air aided by its boundary along with massive traffic becomes the major factor in rise of temperature inside of tunnel along with humidity as water drops trap inside of it due to lesser air currents.

**HABITAT CHARACTERISTICS AND PREFERENCES OF HIMALAYAN IBEX
(CAPRA IBEX SIBIRICA) IN KARAKORUM RANGE OF PAKISTAN****Ghulam Raza, Maqsood Anwar, Sarwat N. Mirza, Azhar Hussain and Rukhsana Tariq**¹*Department of Biological Sciences, University of Baltistan, Skardu, Pakistan*²*Department of Wildlife, PMAS-Arid Agriculture University Rawalpindi, Pakistan*³*Department of Forestry and Range Management, PMAS-Arid Agriculture University Rawalpindi,*⁴*Department of Environmental Science Karakoram International University, Gilgit-Baltistan,*⁵*Department of Food Science and Technology Karakoram International University Gilgit-Baltistan,*⁶*Department of Environmental Sciences IIU, Islamabad Pakistan**Corresponding Author: ghulam.raza@kiu.edu.pk, ghrazabalti@yahoo.com

Himalayan ibex (*Capra Ibex sibirica*) is a subspecies of *Capra Ibex*, and is evenly distributed throughout the upper comparatively dry mountains of Gilgit-Baltistan, Pakistan. It is categorized as a *Least Concern* species internationally as well as in Pakistan, however its presence in the karakorum mountain plays significant role in the economy of the region as dozens of trophy hunting of this specie takes place in the region every year and generates handsome money to the local inhabitants of the mountainous community. The characteristic habitat analysis and preferences was important as the ibex move downwards during winter to cope the fodder shortage after heavy snowfall. This may expose the ibex to potential threats of predation by predators, poaching and competition for food with livestock during winter season. Therefore, the study was conducted to analyze the characteristic habitat feature and preferences of Himalayan ibex during winter season in Hushe valley of Karakorum range. The study was conducted in the winter season from 15 November 2012 to 15 February 2013. The duration of the study period was constrained by the remoteness and inaccessibility of the study area. Two permanent transects one was 7 km in length upstream from Hushe village (centre point) to Dumsun Junction and the second was 5 km long downstream from Hushe village (centre point) to Hushe bridge were established along the Hushe river. Twelve major locations and three vantage points on each location were identified based on abundance of ibex population. Each trail was walked nine to ten times during the whole study period. On each time, walk observations were made on every three vantage points of twelve locations. The total of 28 observations on average were taken. The Field observations were aided by 8x40 binoculars and a 15-45X

spotting scope. Each and every time a group of animals was encountered, the time, date, species, number and habitat characteristics such as distance to the cliff, slope angle, and elevation snow cover % was recorded in the designed format. The association of ibex with respect to habitat characteristics were calculated by taking the number of observation out of total twenty-eight observations. All habitat variables were visually estimated except elevation, which was determined by using a Global Positioning System (GPS), model E Trex 30. For estimating the proportions of available habitat, Google Earth Pro was used to find out the total estimated covered area of the ibex habitat, with the help of GIS experts at the WWF office in Gilgit. The whole valley was sampled for the study of biomass cover and productivity in another study by the author; therefore, the habitat characteristics were easily noted during the survey in winter. The total area was estimated as 172 Km² with Google Earth Pro. In 25% observation ibex was found on slopes with partial snow cover, followed by 21% on cliffs and in 14% observations the ibex were found on areas fully covered with snow. Only 2% of the observations were on scree and barren areas. There was a significant difference in use of different habitat types. Ibex showed a preference for sloped areas with a snow cover habitat. Almost 50% of the observations of ibex were close to the cliffs, while only 21% of the observations were away from cliffs. There was a significant preference of ibex to live close to cliffs (25-50 m). Table 1.3 shows that 53% of Himalayan Ibex were observed on steep slopes (70-80). 32% were observed on moderate (60-70) slopes and there was a significant difference in use of different slope angles. In the present study, 42% of the ibex were observed in between 3200-3400 m altitude and there was no significant difference in use of altitudinal variations. There was a significant difference in use of habitat types by the ibex. The high preference was shown towards slopes with partial snow cover habitats which showed link and the affinity of Himalayan Ibex for steep rugged terrain to avoid predation. The distribution with respect to cliff and steep slope might be to avoid being preyed on by snow leopards. In the present study, the ibex comes to lower altitude for searching of food. Snow cover and lack of food in snowy areas forces them to seasonally change their home ranges. Therefore, there might be an increased risk of predation by snow leopard and poaching by hunters. The downward movement in winter may also cause shortage of food for domestic livestock and competition may occur among them. The results of the study will be helpful for conservationist for planning of conservation strategies.

SEASONAL VARIATION IN POPULATION STRUCTURE OF HIMALAYAN IBEX (*CAPRA IBEX SIBIRICA*) IN KARAKORAM MOUNTAIN RANGE, PAKISTAN

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Himalayan ibex (*Capra ibex sibirica*), wide spread in northern Pakistan, contributes significantly to sustaining predators populations and earning revenues for local communities through trophy hunting. Lack of information about population dynamics is a daunting challenge in devising long-term conservation strategies for *C. sibirica* in and around Protected Areas of northern Pakistan. The study was conducted in five catchments of the Central Karakoram, during spring (May) and winter (December) 2013, by fixed-point direct count method using specific vantage points from where animals could easily be sighted during dawn and dusk and the same points were used in the subsequent surveys. We used binoculars (Nikon 12 x 50) and spotting scope (Swarovski ATM 80 HD) to count animals; a hand-held GPS (Garmin 78) was used to record locations and elevation of vantage points and a compass to note down bearings (angle), while distance from vantage points to location of herds was estimated approximately. SPSS 20 was used to analyze data. In addition to mean group size, typical group size was also calculated based on animal-centered measurements by squaring the sizes of groups, summing across all groups and dividing the sum by total number of individuals observed. Density was estimated using total counts and the surveyed area. Mann-Whitney U test was applied to compare reliability of sample means between different survey timings (seasons). Kruskal-Wallis H test was used to determine difference in sex ratio between different density areas. Pearson chi-square test was used to compute ratios among different sex and age classes. A three-way analysis of variance with valleys/area, habitat type and

weather was used to examine whether typical group size differed under the influence of any of the factor or interaction among factors. To measure the effect of age class within as well as against weather and habitat type on Ibex population distribution, we used spearman correlation (r). Also linear regression (R^2) was used to look for the relationship between group size and density. **Results:** Over 2,859 ibex were observed in five catchments including 1143 in spring and 1716 in winter. Males were 33% and 27%, females were 38% and 37%, yearlings were 18% and 11% and kids were 8% and 20% in spring and winter populations, respectively. In addition trophy size males were 14% and 8% and non-determined individuals were 4% and 6% in spring and winter populations, respectively. Sex ratio between male and female decreased significantly across spring to winter ($\chi^2 = 10.0$, $df = 9$, $P = 0.350$). Whereas, ratios between kids to female increased ($\chi^2 = 8.00$, $df = 8$, $P = 0.433$) and yearling to female increased significantly ($\chi^2 = 10.0$, $df = 9$, $P = 0.350$). Increase in an average typical group size, while no significant change in mean group size was observed across both seasons. Average population density of Himalayan ibex in central Karakoram was 3.24 ibex per ($\times 10$) km^{-2} , with highest typical group size and density in Hisper 4.64 km^{-2} followed by Hoper (4.25 km^{-2}) and Thalay (3.47 km^{-2}) and lowest in Hushey (2.0 km^{-2}) and Basha (1.76 km^{-2}). Result of three way and two way ANOVA between weather, habitat type and season indicated no significant effect of either of these factors on mean group size of ibex in CKNP. A strong correlation was observed among typical group size and habitat type ($r = 0.286$, $P = 0.000$), whereas no significant correlation was observed between weather and typical group size. Whereas, a positive correlation was observed among habitat type and weather ($r = 0.296$, $P = 0.000$), indicating that weather is somehow indirectly affecting the typical group size. Population structure of H. ibex in central Karakoram varies with seasonal variations. Understanding seasonal variation is important to determine impacts of various topographic, climatic, edaphic and associated factors on habitat types leading to varied population structures of wild ungulates. A strong correlation between weather and habitat type indicate that weather is indirectly affecting the ibex group size in addition to habitat type.

**NEW DISTRIBUTION RECORD OF HIMALAYAN WOLF SNAKE
(*LYCODON MACKINNONI* WALL, 1906) IN AZAD KASHMIR (PAKISTAN)**

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The present study was conducted to document house killed mortality of ophoid fauna in rural areas of (Bagh) Azad Jammu Kashmir. The survey was conducted from June 2016 to June 2017. During survey, we collected 3 samples of wolf-snake species (*Lycodon mackinnon*) by using the visual encounter method. The samples were collected from three sites, (Panyali, Chamman kot, Dnna) in the area of and their morphometric, Pholidotic count were done by using taxonomic keys. These findings are new extension in distribution range of the species (*Lycodon mackinnon*) Azad Kashmir because First distribution record in Azad Kashmir, was documented (Faiz et.al.2017 Amphibian & Reptile Conservation).

**DISTRIBUTION, HABITAT USE AND SOME ASPECTS OF BREEDING BIOLOGY OF BLACK
FRANCOLIN (*FRANCOLINUS FRANCOLINUS*) IN PIR LASURA NATIONAL PARK,
KOTLI, AZAD JAMMU AND KASHMIR**

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Black francolin (*Francolinus francolinus*) is a game bird. Belonging to Order Galliformes, Family Phasianidae, Sub-family Perdiciinae and Genus *Francolinus*. Black francolin is considered as Least Concern globally as well as in Pakistan. Pir Lasura National Park (PLNP) falls in distribution range of black francolin in

Azad Jammu and Kashmir. Present study was conducted to determine the distribution, habitat use and breeding biology of this bird in PLNP. Study sites were selected through reconnaissance surveys and on the basis of information collected from local people, shepherds and wildlife staffs. To determine the distribution of black francolin, study area was divided into five localities (Karela, Dabsi, Pir Kalengar, Khandhar and Pir Kane) on the basis of direct and indirect evidences. Selected sites were surveyed early in the morning and evening, when this species was more active. The results indicated that black francolin is distributed in all localities and preferred high altitude between 1200 to 1800 m elevation. The encountered rate and calls of black francolin were maximum in locality Karela (n=16), Pir Kalengar (n=18) and Khandhar (n=10) and minimum in Dabsi (n=5) and Pir Kane (n=4). For habitat analysis of the species phytosociological surveys were conducted in each selected study sites using quadrat method. A total of 57 plant species were recorded in five potential habitat of black francolin. The composition of vegetation was trees (28.07%), shrubs (28.07%) and herbs and grasses (43.85%). The dominant vegetation was *Pinus roxburghii*, *Acacia nilotica*, *Acacia modesta*, *Olea ferruginea*, *Dalbergia sissoo*, *Pinus wallichiana*, *Eucalyptus camaldulensis*, *Punica granatum*, *Carissa opaca*, *Indigofera heterantha*, *Rosa brunonii*, *Berberis lyceum*, *Cynodon dactylon* and *Heteropogon contortus*. There is no significant difference between vegetation of five study sites ($\chi^2=1.088$, df=4, $p>0.05$). Breeding season of black francolin in the study area started from mid-May to July. Four nest were observed during survey, three in forest and one in cultivated field. The clutch size was 8-10 eggs per nest and incubation period was 18 days. Comparison of breeding parameter in both forest and cultivated habitat revealed a non-significance difference among Diameter of Nest ($t=-1.3$; $p=0.11 > 0.05$), Incubation Period ($t=-2$; $p=0.058 > 0.05$) and weight of egg ($t=0.32$; $p=0.38 > 0.05$). Future conservation efforts for black francolins in PLNP Park, need to focus on protection and securing disturbance-free habitat.

HUMAN-COMMON LEOPARD (*PANTHERA PARDUS*) CONFLICT IN LACHHRAT FOREST RANGE, MUZAFFARABAD, AZAD JAMMU AND KASHMIR, PAKISTAN

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Human-carnivore conflict intensified to a large scale in recent decades. Common leopard (*Panthera pardus*) is one of the widely distributed big cats in Pakistan and AJ&K. Increasing leopard killing incidents in AJ&K indicate the rise in conflict with human beings. Present study aimed to measure this conflict in Lachhrat Forest Range, situated between Neelum and Jhelum valleys of AJ&K. Data were collected during January, 2016 to December, 2017 through field surveys and questionnaires asked to the local community. A total of 13% of the respondents claimed that they have seen common leopard in their vicinity in their lifetime. Data revealed that a total of 108 livestock were killed by common leopard, highest depredation was noted on goats (46.29 %) followed by sheep's (29.62 %) while minimum depredation was recorded on equine (0.92%). Most of the livestock were killed in April (26%) followed by May (22%) and June (18%) whereas minimum 2% depredations were noted in December, heightened depredation to 58% in summer as compared to lowest 4.6% in winter season. Overall a total of 70% attacks were recorded at night. Most (62%) of the people did not adopt any anti-predation strategy, however, 14% of the respondents reared watchdogs. Least 2% people used fire and marriage bombs to keep the leopard away from their pens or livestock. Overall, loss was estimated up to 1.92 million PKRs to the local people during study period. Such losses generate negative attitude among the local herders. Half (45.79%) of the respondents replied that the conservation of the leopard is not necessary, while 2% of the respondents admitted that they have killed common leopard. However a small percentage (2%) of respondents replied that it should be conserved because of its ecological role and the context of biodiversity conservation. Awareness regarding improvement in herding practices, better watch and ward conditions, use of frightening devices could reduce conflict intensity and hence it would bring a positive change in the attitude of local people toward the conservation of common leopard. This study can be compared to other regional investigations and it provides a step toward the conservation efforts of this species in Lachhrat Range Forest.

THE NEW RECORD OF *GONIODES* NITZSCH, 1818 (PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) FROM *COTURNIX JAPONICUS* (GALLIFORMES: PHASIANIDAE) IN SINDH, PAKISTAN

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Ectoparasites are the external obligate parasites which are found practically on the outer body parts of animals. *Coturnix*, commonly known as Quail or Batair is a popular and economically important bird of avian order Galliformes in Pakistan. This study has been carried out on the chewing lice infestation in the *Coturnix japonicas*, for the first time in Sindh, Pakistan. During present survey, one species, *Goniodes astrocephalus* (Burmeister, 1838) was reported from *C. japonicas* for the first time in the host from Pakistan, making new host and new locality record in the world.

PREVALENCE OF CHEWING LICE ON COOTS (GRUIFORMES: RALLIDAE) FROM MAJOR WATER BODIES OF SINDH PAKISTAN

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Coots are sharpest, aggressive and extremely active birds of order Gruiformes. They feed vigorously on small crustaceans, aquatic insects and small fishes as well as on aquatic weeds, having impressive number of endoparasitic infection in it. This study has been carried out for the ectoparasitic infestation in coots in Sindh region for the first time, with special reference to the prevalence and rate of infestation in it. Total 80 birds were examined for their chewing lice infestation in which 70 birds went positive with 87.5% prevalent for chewing lice and 30 birds were infested with mites having 37.5% prevalence. More than 1200 lice and more than 280 mites were recovered during present study from different regions of Sindh, Pakistan.

STUDY OF WILD BOAR (*SUS SCROFA*)-HUMAN CONFLICTS IN ABBASPUR, AZAD JAMMU AND KASHMIR, PAKISTAN

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The study was conducted to assess the wild boar - human conflict in Abbaspur area of Azad Jammu and Kashmir from April to December 2016. A total of 28 surveys were conducted to collect information about crop damage or raiding by wild boar. Data on crop raiding and attitude and tolerance of community towards this animal was collected through questionnaire survey (n=200). Main crop damaged by wild boar was maize however in small extent vegetables were also damaged. An area of 532.5 hectares and 44.96 metric tons of maize was damaged during the last three years. Based on local market price, the value of the damage was approximately Rs. 1.3488 million Pakistani rupees. Three persons were injured during the last 3 years by wild boar. In response, during the last three years, three wild boars were killed by the community. Accordingly, 79% (n=158) of respondents dislike wild boar, while 21 % (n=42) were in favor of wild boar conservation. About 85% (n=170) respondents recommended that wild boar should be conserved in zoos and wildlife parks, while 15% (n=30) recommended has conserve them in natural habitats. Wild boar population density increased in the study area. During 2016, the population density of Wild boar was estimated as 9.63/km². Currently the methods used to minimize crop damage by wild boar include physical barriers, human puppets and effigies, electric fences and manual surveillance, which are almost ineffective or very expensive.

EXPLORING THE WILD MAMMALIAN DIVERSITY OF DISTRICT TOR GHAR, KHYBER PAKHTUNKHWA, PAKISTAN

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The study survey was conducted from May 2016 to May 2017 about the wild mammalian diversity of district Tor Ghar KP. During the research ten species belongs to 8 families, 6 orders, 10 genera of mammals have been reported. The reported species are *Naemorhedus goral*, *Presbytis entellus*, *Macacamulatta*, *Myotis myotis*, *Sus scrofa*, *Panthera pardus*, *Lepus nigricollis*, *Canis lupus*, *Hystrix indica* and *Canis aureus*. During the research Grey langur, Monkey, Porcupine, Hare, Wild boar and Jackal species are abundant. Due to habitat destruction, deforestation, over hunting and urbanization, the Leopard and Wolf species are becoming endangered.

EXPLORING THE WILD AVIAN DIVERSITY OF DISTRICT SHANGLA, KHYBER PAKHTUNKHWA, PAKISTAN

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The present study was conducted from May 2016 to May 2017 to explore the Wild Avian diversity of district Shangla Khyber Pakhtunkhwa Pakistan. As a result of this study 12 different species were recorded belonging 12 genera 5 families and 5 orders. The order Galliformes were more in number. The recorded species include, *Alectoris chukar*, *Ammodramus griseogularis*, *Francolinus francolinus*, *Lophura leucomelana*, *Lophophanes impejanus*, *Pucrasia macrolopha*, *Columba livia*, *Otospilocephalus*, *Gruidae grus*, *Ictinaetus malaiensis*, *Columba leuconota* and *Aegypius bengalensis*. During the study direct and indirect method were used and visit the field for collection of information from the local community and hunters about the present and past status of wild avian diversity of district Shangla. During the research Chukar, Koklas pheasant, Rock pigeon, Mountain scops Owl and Vulture were abundant. These species were found in every localities of the study area. Black partridge, See See, Kalij pheasant. Monal pheasant and Snow pigeon were found rare.

PUBLIC PERCEPTION ABOUT WILDLIFE CONSERVATION IN MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN

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We conducted present study from July 2016 to October 2016 to collect data about perception of local people about wildlife conservation in and around protected area, Machiara National Park (MNP), Muzaffarabad Azad Jammu and Kashmir. We collected data through semi-structured questionnaires (n=80) and group discussions targeting permanent and temporary residents of the area. Local people depend upon livestock, crops, forest for timber, fuel wood and medicinal plants. Data analysis showed that majority (70%) of the respondents had negative perception about wildlife conservation due to damage caused by the wildlife in terms of livestock depredation, crop raiding and human casualties. Respondents bear losses of maize crop in the form of both grains and fodder. Other crops included wheat, corn and fruits. The common animals that destroyed the maize crop were bear, pig, monkey and porcupine. Besides crop raiding, wild animals also preyed on livestock. However, 30% (n=24) of respondents were in favour of conservation of wildlife in the study area. It was concluded that majority of the local people are against conservation of wild animals due to their vested

interests. It is suggested that an awareness programme should be launched to educate locals about the importance and roll of wild animal in healthy ecosystem.

COEXISTENCE OF HIMALAYAN MUSK DEER (*MOSCHUS LEUCOGASTER*) AND DOMESTIC UNGULATES IN MACHIARA NATIONAL PARK, PAKISTAN

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Himalayan musk deer (*Moschus leucogaster*) is a globally threatened (Endangered) species, still existing in the small pocketed populations in the northern mountainous regions of Pakistan and Azad Jammu and Kashmir, including Machiara National Park. However, due to various anthropogenic activities and heavy livestock pressures, its population is under severe threats in all these areas. A study was conducted to assess the current distribution, habitat utilization and habitat and diet overlapping between musk deer and domestic livestock (sheep/goats) in Machiara National Park during April to November 2014. For this purpose, direct (physical observation and signs in sampling plots; n=60) and indirect (information through shepherds, hunters and field staff) methods were used to collect field data. Musk deer were found in 18 different localities of the study area at the elevation range between ~2450-4000 m above mean sea levels (asl). A total of 41 plant species were recorded from musk deer habitat in the study area comprising trees (n=9), shrubs (n=8), and herbs/grasses (n=24) with 21.95%, 19.51%, and 58.94%, relative frequency, respectively. The dominant vegetation associations included *Abies-Viburnum-Polygonum* community, *Taxus-Indigofera-Artemisia* community, *Betula-Rhododendron-Ajuga* community and *Berberis-Rosa* community. Overall, in musk deer habitat, *Abies pindrow* (IVI=38.17), *Picea smithiana* (IVI=28.97), *Pinus wallichiana* (IVI=27.46), *Betula utilis* (IVI=22.02), *Viburnum nervosum* (IVI=22.04) and *Rhododendron campanulatum* (IVI=19.85) were the dominating species among vegetation. Musk deer mostly preferred sub-alpine scrubs (IV=0.11) and mixed coniferous forests (IV=0.09) with moderate (IV=0.46) and sparse (IV=0.36) vegetation, in eastern (IV=0.07) and southern (IV=0.03) aspect, with steep slopes (51° to above 70°) and ridged terrain (IV=0.31). About 73.4% (J=0.42, <0.05) of musk deer habitat was overlapped by the domestic goats and sheep in Machiara National Park. Total of 28 plant species were recorded as the diet of musk deer, out of these, 20 plant species were also consumed by sheep/goats, indicating a huge overlap of about 71.43% (C_λ=0.897) in the diets of musk deer and domestic goats and sheep. This high overlapping between the habitat and diets of both animal groups and habitat destruction by the livestock is posing the serious threats to the population of musk deer in Machiara National Park.

A STUDY OF BIRD DIVERSITY IN DIFFERENT LOCALITIES OF DISTRICT HAVELI AZAD JAMMU AND KASHMIR (PAKISTAN)

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Present study was conducted to study Avian diversity from March to November 2015 in Palangi, Lasdana and Shearodhara in district Haveli Azad Jammu and Kashmir. The area was surveyed in the morning and evening when the birds activity is at peak. Birds were recorded by walking predetermined Line-transects of various sizes at different selected sites. Data were recorded using binocular and digital camera and identified with the help of field guide by Grimmett et al (2008). A total of 58 species, belonging to 25 families and 10 orders were recorded in the study area. Results showed that bird species belonging to order Passeriformes were the most abundant (55%) followed by Piciformes (8%) Apodiformes (7%) Strigiformes (5%) and Coraciiformes (2%). The distribution, abundance and species richness varied with seasons. Prominent bird species adapted to diverse habitat of Palangi, Lasdana and Shearodhara and its surrounding included *Terpsiphone paradise* (relative abundance 0.0309), *Apus melba* (0.03763), *Acridotheres tristis* (0.0873), *Corvus splendens* (0.1357), *Parus major* (0.0094), *Passer domesticus* (0.1290), *Pycnonotus leucogenys* (0.0584), *Dendrocitta vagavunda*

(0.0114), *Athenebrama* (0.0020), *Pica nuttalli* (0.1055), *Pycnonotus cafer* (0.0040). Species richness was recorded highest in spring (n=34) and lowest in autumn (n=30). Given the diverse habitat, species richness and avian diversity of the area, it is timely to protect the area. It is suggested to further enrich the habitat by increasing plantation that will ultimately increase habitat diversity, food, nesting sites and other requirements for the bird species.

EFFECT OF FEEDING ON GROWTH OF RING-NECKED PHEASANT (*PHASIANUS COLCHICUS*)

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Ring-necked pheasant is a beautiful bird and reared as pet in many countries. Nevertheless, knowledge about the feeding regimens on growth of chicks is lacking in published literature. Therefore, present study was designed to evaluate the effect of feeding regimens (mash, crumble and pellet) on the growth of ring-necked pheasant chicks. One hundred day old chicks were divided into three groups and fed on three different types of feed. Observations were recorded at different stages of growth viz; starter (0-30 days), grower (31-90 days) and finisher (90-120 days). The data on weight gain, feed conversion ratio, performance index, survivability and production number were recorded. Higher ($P<0.05$) weight was recorded in chicks maintained on mash feed compared to pellet and crumble feed. Chicks at starter age have more ability to convert feed into live weight gain compared to chicks at grower and finisher age. However, the chicks maintained on crumbled feed have higher ($P<0.05$) FCR compared to mash and pellet feed. The chicks showed high performance index with mash feed as compared to the crumble and pellet feed. At starter stage, crumble feed showed the highest ($P<0.05$) production number compared to pellet and mash feed. At grower stage, mash feed showed the highest ($P<0.05$) production number than crumble and pellet group. At finisher stage, production number was highest ($P<0.05$) with mash feed compared to crumble group and pellet group. It is concluded that mash feed is the more beneficial for ring-necked pheasant for growth and rearing.

INFLUENCE OF GROUP SIZE AND ENVIRONMENTAL CONDITIONS ON BREEDING PERFORMANCE OF INDIAN PEAFOWL (*PAVO CRISTATUS*) IN CAPTIVITY

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Indian peafowl (*Pavo cristatus*) is polygamous bird and kept as pet in many parts of the world as well as Pakistan. Under captive environment, group size, caging environment may affect the breeding success and the birds develop stereotypic behaviors due to stressful captive environment. Therefore, present study was designed to investigate the breeding biology and time budgeting of Indian peafowl in four captive breeding centers (site 1: Bahria Safari Park, site 2: Ayub Park, site 3: Lohi Bheer Wildlife Park, site 4: Mehria Avian Research Station). A total of 250 Indian peafowl (125 males, 125 females; sex ratio, 1:1) at Bahria Safari Park, 120 birds (30 males, 90 females; sex ratio, 1:3) at Ayub Park, 60 birds (15 males and 45 females; sex ratio, 1:3) at Lohi Bheer Wildlife Park and 6 birds (3 males, 3 females; sex ratio, 1:1) at Mehria Avian Research Station were studied. Time budget analysis shows that Indian peafowl spent most of the time in sitting (44.1 %) followed by standing time (14.4 %), display time (10.4), feeding time (10.1), preening time (8.5 %), mounting time (1.6 %) and very less flying time (0.5%) at all study sites. However, maximum eggs were laid in Bahria Safari compared to Lohi Bheer Wildlife Park, Ayub Park and Mehria Avian Research Station. Maximum no. of fertile eggs and percent fertility, hatchability of the fertile eggs were highest in Bahria Safari Park compared to all other study sites. Indian peafowl is a social bird, live in large groups and maintain its territory in nature. Influence of group size and sex ratio was most favored at Bahria Safari Park compared other study sites. It is

concluded that Bahria Safari Park is providing better captive conditions due to manpower, maintenance, cleaning and may play role in the conservation of this beautiful bird.

PREPARATION OF CHECKLIST OF BREEDING BIRDS OF MUZAFFARABAD CITY, AZAD JAMMU AND KASHMIR, PAKISTAN

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Birds are indicators of healthy ecosystem. Present study was conducted to compile a checklist of breeding birds of Muzaffarabad city. Sum total of 15 study surveys were conducted during the breeding season (April to November, 2016) of avian fauna from different localities of Muzaffarabad. The data was collected using line transect and point count methods. Relative abundance and Shannon Wiener diversity index was calculated. A total of 31 bird species were recorded from the study area during the study period. Among 31 recorded species, 22 were breeders and 9 were winter visitors. The breeding birds belonged to 8 orders and 19 families. Among 22 recorded species of breeding birds, the dominant family of the area was Pycnonotidae (n=3) followed by Corvidae and Sturnidae (n=2 each). Maximum diversity was seen in Chehla Bandi (2.810) followed by Plate (2.654), Tariqabad (2.291), Jalalabad (2.280), Main City (2.272) and Sathra (1.992). Local people should be encouraged for plantation in the Muzaffarabad city and its surroundings that will increase habitat diversity and species richness of breeding bird of the area.

PHYLOGENETICS AND HAPLOGROUP DIVERSITY OF SHEEP BREEDS OF KHYBER PAKHTUNKHWA

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The livestock has a key role in agriculture sector to get valuable products. Animal production continues to play a pivotal role economically and culturally in many rural communities. Domestic sheep is quadrupedal, ruminant mammal and make a major contribution in livestock sector. The study was carried out on sheep breeds from Khyber Pakhtunkhwa. Blood samples (n=487) were collected from 10 sheep breeds. The extraction of DNA was carried out from frozen samples following the organic extraction method using Chloroform and phenol mixture. The mitochondrial DNA from 159 blood samples was amplified by Polymerase Chain Reaction. After cleaning nested PCR product it was placed in genetic analyzer for sequencing of the DNA samples. The sequenced data was aligned with already identified sheep mtDNA haplogroup reference sequences to assign the haplogroups to which the analyzed sheep breeds belonged. The Phylogenetic results showed the presence of three haplogroups (HapA, HapB and HapC) in the 159 analyzed samples. The haplogroup described in the literature (HapD and HapE) were not found. The results showed that 124 out of 159 sequences were clustered with Haplogroup A (77.99%), 30 were clustered with haplogroup B (18.87%) whereas 5 tested sheep was clustered with haplogroup C (3.14%). Among them haplogroup A was predominant (77.99%) followed by haplogroup B (18.87%) and C (3.14%). One hundred and six haplotypes were observed from 159 mtDNA loop sequences of sheep breeds of K.P corresponding to 3 haplogroups. The Phylogenetic analysis showed that most of the breeds clustered with two wild sheep; *Ovis ammon* and *Ovis orientalis anatolica*.

NEW RECORD AND BASELINE STUDY OF PALLAS'S CAT *OTOCOLOBUS MANUL* IN PARACHINAR, KURRAM AGENCY, PAKISTAN

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Baseline studies are essential to explore the biodiversity in regions that potentially harbor globally important and threatened species. In 2013, the Pallas's Cat *Otocolobus manul* has been recorded in Qurumber National Park, but to date nothing is known about its distribution in other mountainous parts of the country. The present study aimed at collecting baseline information about this elusive small wild cat in Parachinar Valley, northwestern Pakistan. During our field survey it was recorded in three localities with photo-captured record. To collect further evidence for the cat's presence in this area, an interview survey was conducted in 23 villages with 230 local people. About half of the respondents sighted a Pallas's Cat in the area regularly. Most of the sightings were reported in forested areas, followed by mountainous and rocky terrain. About 25% of all respondents were aware of the species's key role in this ecosystem as a predator of rodents and lagomorphs, e.g. Pika *Ochotona*. Respondents reported retaliatory killing of five individuals between 2012 and 2017, and capture of three individuals for local trade. Habitat encroaching, poaching and retaliatory killing by local people are potential threats to the Pallas's Cat population in the environs of the Parachinar Valley. Further surveys in potentially suitable habitat are urgently needed to determine its presence and conservation status. Such data will allow for developing an adequate conservation strategy.

DIVERSITY AND DISTRIBUTION OF AMPHIBIAN AND REPTILES IN CHOLISTAN DESERT, PAKISTAN

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Present one year survey was conducted from May, 2016 through April, 2017 to assess the herpetofaunal diversity at Cholistan desert in district Bahawalnagar, Punjab, Pakistan. District is administratively divided into five tehsils viz. Chishtian, Haroon Abad, Minchin Abad, Fort Abbas and Bahawalnagar. Field surveys were conducted during dawn and dusk hours for fifteen consecutive days in alternate months and 5 sub sampling sites were sampled in 10 field visits. Specimens were collected through bare hands, using snake sticks, forceps, drag nets, noose, pitfall and funnel traps. Overall, 2 toads, 2 frogs, 2 turtles, 10 lizards and 10 snakes' species were recorded belonging to 23 genera and 14 families. Simpson index was calculated as 0.9334, Evenness 0.733 and Shannon wiener index as 2.947 indicating moderate to high level of diversity. *Bufo stomaticus* (Pi=0.1253, *Uromastyx hardwickii* (Pi=0.0739) were dominated amphibian and reptilian species respectively while *Uromastyx asmusi* was recorded first time from the study area. Hand picking and pitfall traps appeared to be the most effective methods to capture the amphibian and reptiles as compared to other sampling techniques.

HERPETOFAUNA OF SALT RANGE, PUNJAB, PAKISTAN

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This pioneer one year survey was conducted from April, 2015 through March, 2016 around five dams in Potwar Plateau of Salt range, District Chakwal, Punjab namely Dhok Tahlian Dam, Dhoke Qutab Din Dam, Khokhar Zair Dam, Surla Dams and Kot Raja Dam. Overall, 31 herpetiles species including 6 amphibians and

25 reptiles' species representing 28 genera, 3 order and 15 families were captured through direct and indirect sampling methods. It has been observed that handpicking along with pitfall traps were the effective methods to capture herpetiles in the study area. Most dominated amphibian and reptilian species were *Hoplobatrachus tigerinus* and *Hemidactylus brooki* respectively. Evenness e^H/S , Simpson and Shannon index was calculated as 0.8305, 0.9557, 3.248 respectively indicating moderate to high level of diversity in the study area. Conservation status of herpetiles is yet to be evaluated and none of these species are protected by law in Pakistan. However, during current survey a rough estimate of populations has been made with 19 species were evaluated as common, 9 species as less common and 2 species were rare. In our recommendation, such regional surveys are needed and molecular analysis should take into consideration for better identification of cryptic and morphologically same species.

**BREEDING BIOLOGY OF LAUGHING DOVE (*SPILOPELIA SENEGALENSIS*)
IN DISTRICT OKARA, PAKISTAN**

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The breeding biology of laughing dove (*Spilopelia senegalensis*) was studied in district Okara, Pakistan. A total of 52 nests were located in the study area, from which 37 nests were found active for breeding activity. The successful nests were positioned on middle of the plants (50%) followed by other position (25%), fork (19%) and terminal (13%). The preferred height for nest construction from ground was 1-2m (80%) followed by 2-3m (14%) and 0-1m (5%). The preferred tree for nest construction was Kikar (*Vachellia nilotica*; 73%) followed by Bamboo (*Bambusa vulgaris*; 13%), Guava (*Psidium guajava*; 11) and Neem (*Azadirachta indica*; 3%). The laughing dove preferred to construct nest on trees at canal bank (73%) followed by parks (16%) and orchids (11%). It was observed that laughing dove laid second clutch in 59% of the nests. However, the hatching and fledgling success is found variable in both clutches and recorded 100% for clutch 1 and 94% for clutch 2. Predation rate of eggs was 27% and only 3% of the eggs were infertile from first clutch. However, 86% of the juveniles were fledged and 14% were fallen out from nest. Although no infertile eggs were recorded in 2nd clutch but egg predation eggs was 18% and other losses were 7%. All of the nestlings were fledged from 2nd clutch. It is concluded that laughing dove is breeding remarkably in the district Okara and prefer to construct nests on *Vachellia nilotica* on the bank of canal.

**A STUDY ON ECOLOGY AND BREEDING BEHAVIOR OF SPOTTED OWLET
(*Athene brama*) IN DISTRICT KASUR, PUNJAB, PAKISTAN**

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Ecology including nesting and breeding behaviors of the spotted owl (*Athene brama*) was studied from the beginning of January to April 2016 and 2017 in Kasur district, Province Punjab, Pakistan. There were found 38 nest sites but out of these 22 located in tree sites and 16 in buildings areas. The trees used by the Spotted Owl for nesting were *Ficus benghalensis* (40.9%), *Dalbergia sisso* (22.7%), *Acacia nilotica* (13.6%), *Ficus religiosa* (18.1%), and *Mangifera indica* (4.5%), that provide more suitable nesting in cavities/holes. There were also located nest sites in other places rather than trees as building pipes (37.5%), building walls (31.2%), cutting banks (25%) and mounds of manure (6.2%). The spotted owlets in tree sites were recorded with larger height of nest sites (m), greater distances to nearest human habitations (m), buildings (m), roads (m), electric lines (m) and light sources (m) than that of the building sites without any significance difference ('t' test, $p < 0.05$). While the spotted owlets were recorded in building sites with greater height of nest locations (m), depth and diameter of holes/cavities (cm), distances to nearest trees (m), agriculture lands (m) and water sources (m) than that of the tree sites without any significance difference ('t' test, $p < 0.05$). Number of holes or cavities per

nest site was differed significantly between tree sites and building sites ('t' test, $p < 0.05$). Clutch size varied between 1 and 3 and means of clutch size and brood size were 2.04 ± 0.74 , 1.76 ± 0.70 respectively with the nesting success 81%. The minimum and maximum length and width of eggs were 23.2 x 31.5mm and 20.3 x 26.7mm respectively. Weight of the eggs varied between 9.0 g and 18.0 g with a mean of 15.3 ± 2.08 . The hatching success and fledging success of the spotted owl were 72% and 81% respectively.

A STUDY ON MORPHOLOGY AND PARASITIC PREVALENCE IN BATS OF DISTRICT NAROWAL PUNJAB, PAKISTAN

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Very recent study was conducted to find the common species of bats in district Narowal, Tehsil narowal, Zafarwal, Shakargarh Punjab Pakistan from 13 March 2016 to 2 February 2017. There are 38 total specimens belonging to two species were captured. In which *Pipistrellus pipistrellus* (n = 21), and *Scotophilus heathi* (n = 17) are included. These two species are first time reported from this study area. The bat fauna of the study area was never documented prior to present investigation. In this study morphometric measurement of external body parameters, cranial and bacular measurements are taken and compared with other literature. Ectoparasite study was made on *pipistrellus* and *Scotophilus*. Almost 13 soft ticks larva were collected on belly, ear base, and neck of the *pipistrellus* and *Scotophilus* bats. Identification was made of all larval ticks and nymphs collected from bats. Ticks were identified as *Argas Vespertilionis*. Almost 7 specimens of ectoparasites (Nycteribiidae) collected from the fur of *Scotophilus heathi* at Zafarwal tehsil district Narowal. Ectoparasites are identified up to family level. These specimens were not identified up to species level. These mysterious ectoparasites of bats (*Scotophilus heathi*) have no wings, three pair of legs, and their body is flattened. Their legs adapted for running but not for jumping. They has rounded abdomen but little pointed at the end of abdomen. Whole body has spines but more prominent at the abdomen and large in siz.

DIVERSITY AND DISTRIBUTION OF AVIAN FAUNA TEHSIL MATTI, SWAT, PAKISTAN

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Survey was conducted to study the diversity and distribution of avian fauna in Tehsil Matti, Swat-Pakistan from April 2016 to August 2017. Point count observations were used to determine diversity and distribution. A total of 21 species belonging to 6 orders and 18 families were recorded. Maximum number of species belong to order Passeriformes (71.5%) belonging to 13 families was represented by 17 species viz; *Corvus splendens*, *Dicaeum erythrorhynchous*, *Hirundo rustica*, *Pycnonotus cafer*, *Dicrurus macrocerus*, *Acridotheres tristis*, *Passer domesticus*, *Motacilla madaraspatensis*, *Lanius vittatus*, *Carpodacus pulcherrimus*, *Regulus regulus*, *Terpsiphone paradise*, *Certhia himalayana*, *Acridotheres ginginianus*, *Corvus splendens*, *Corvus corone*, *Acridotheres tristis* and *Acridotheres ginginianus*. Only two species *Lymnocyrtus minimus*, *Limosa lopponica* belongs to order Charadriiformes (9.5%) and only one species *Ardea alba modesta* belongs to order Pelecaniformes (4.70%), Columbiformes (4.70%) by *Streptopelia decaocto*, Coraciiformes (4.70%) by *Acedo althis* and order Falconiformes (4.70%) has been represented by *Buteo rufinus*. Among these species, 47.6% species were resident and 42.9% species were summer migrant and 9.5% were winter migrant. The relative abundance of the birds showed that maximum number of birds were insectivorous (42.8%) followed by insectivores-carnivores (23.8%), granivores (19.0%) and omnivores (14.2%). It is concluded that Tehsil Matti, Swat-Pakistan is rich in avian fauna and providing habitat for the resident as well as summer and winter migrants.

AMPHIBIAN ASSEMBLAGE OF THREE FOREST TYPES OF PAKISTAN

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We undertook the present study to document amphibian assemblage in sub-tropical scrub forest (STSF), sub-tropical pine forest (STPF) and Himalayan moist temperate forest (HMTF). We employed standard time-constrained survey method-visual encounter survey- to document amphibian species diversity in the forest types of Rawalpindi, Islamabad Capital Territory and Ayubia, KPK, during spring and summer seasons of 2014 and 2015. We recorded a total of nine amphibian species: Indus Valley Toad (*Duttaphrynus stomaticus*), South-east Asian Toad (*Duttaphrynus melanostictus*), Ant Frog (*Microhyla ornata*), Hazara Torrent Frog (*Allopaa hazarensis*), Skittering Frog (*Euphyctis cyanophlyctis*), Cricket Frog (*Fejervarya limnocharis*), Murree Hills Frog (*Nanorana vicina*), Bull Frog (*Hoplobatrachus tigerinus*) and Indian Burrowing Frog (*Sphaerotheca breviceps*). We recorded two unique species viz. Murree Hills Frog and Hazara Torrent Frog from sub-tropical pine forest (STPF) and Himalayan moist temperate forest (HMTF). We attribute high elevation with associated high precipitation and low temperature, and freshwater torrent springs to this relatively high endemism the two forest types. We have provided data on distribution and morphometric measurements of anurans of the study area. We found that egg mass count, calling index and tadpole count can be used for monitoring of populations of anuran species of the area except Hazara Torrent Frog and Murree Hills Frog for which only tadpole count is recommended. We suggest regular monitoring of anuran populations, particularly endemics and inclusion of management of their habitat at landscape level in future development projects of the area.

SOME OBSERVATIONS ON ROLE OF ANURAN TADPOLES AS BIOLOGICAL CONTROL OF DENGUE LARVAE UNDER LABORTARY CONDITIONS

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The present study aimed to study consumption of dengue mosquito larvae by anuran (Common Skittering Frog and South-east Asian Toad) tadpoles inhabiting Rawalpindi and Islamabad and to compare consumption of dengue mosquito larvae. The anuran tadpoles were collected from ponds and pools using dip nets. The tadpoles were transferred to buckets containing mixture of tap water and pond/pool water, and brought to laboratory of Wildlife Management, PMAS AAUR. Six trials were carried with anuran species of Common Skittering Frog and South-east Asian Toad. Each trial was replicated thrice. Six anuran tadpoles were transferred to the beakers placed under glass cage and provided with the following combinations: Trial I: Tadpoles fed with dengue larvae only; Trial II: Tadpoles fed with dengue larvae and snail meat; Trial III: Tadpoles fed with dengue larvae and earthworm meat; Trial IV: Tadpoles fed with dengue larvae and beetles; Trial V: Tadpoles fed with dengue larvae and flies and Trial VI: Tadpoles fed with dengue larvae, beetles and snail meat. The observations were made after 24 hours, and the number of consumed dengue larvae was noted. The comparison of consumption of dengue larvae among different trials was made using Kruskal-Wallis test. The comparison of consumption of dengue larvae between the two anuran species was made using Wilcoxon test. The results of trial I showed 50, 100 and 75% consumption of dengue larvae by tadpoles of Common Skittering Frog (*E. cyanophlyctus*) in replication 1, 2 and 3, respectively. The trial II showed 75, 100 and 50% consumption in replication 1, 2 and 3, respectively. The trial III showed 50, 100 and 75% consumption in replication 1, 2 and 3, respectively. The trial IV showed 100, 100 and 75% consumption in replication 1, 2 and 3, respectively. The trial V showed 50, 100 and 75% consumption in replication 1, 2 and 3, respectively. The trial VI showed 100, 100 and 100% consumption in replication 1, 2 and 3, respectively. The Kruskal-Wallis test showed that the number of consumed dengue larvae among various trials did not differ-significantly ($P=0.48$). The results of trial I showed 100, 100 and 100% consumption of dengue larvae by tadpoles of South-east Asian Toad (*D. melanostictus*) in

replication 1, 2 and 3, respectively. The trial II showed 75, 75 and 100% consumption in replication 1, 2 and 3, respectively. The trial III showed 75, 100 and 75% consumption in replication 1, 2 and 3, respectively. The trial IV showed 100, 100 and 75% consumption in replication 1, 2 and 3, respectively. The trial V showed 75, 100 and 75% consumption in replication 1, 2 and 3, respectively. The trial VI showed 100, 100 and 100% consumption in replication 1, 2 and 3, respectively. The Kruskal-Wallis test showed that the number of consumed dengue larvae among various trials did not differ significantly ($P=0.26$). The Wilcoxon test showed that the number of consumed dengue larvae in various trials by tadpoles of the two anuran species did not differ significantly ($P=0.08$).

DIVERSITY AND CONSERVATION OF WILDLIFE IN KALA CHITTA NATIONAL PARK

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Kala Chitta National Park is located in district Attock, at N 33°39.617 and E 72° 25.873, elevation ranges from 500 m to 2100 m, covering an area of 36,965 ha. It is one of the largest hilly ranges in Pothwar Region of the Punjab and was declared a National Park in 2008 with the specific objective to conserve Punjab Urial (*Ovis vignei punjabiensis*). Climate of study area is harsh with mean minimum temperature of 17.92°C in January and mean maximum temperature of 41°C in June. Rainfall pattern is scanty and uncertain with mean annual rainfall of 605 mm. Present study was conducted to establish baseline data on wildlife and its habitat, threats to wildlife and its habitat and suggest measures for its conservation. Appropriate scientific methods were used to observe and record floral and faunal diversity. During the study, 9 mammals, 80 birds, 9 reptiles and 7 amphibian species were recorded from the park. Major wildlife species of the park included Punjab Urial, Indian Wolf, Indian Pangolin, Chukar Partridge, Grey Francolin, Black Francolin, Himalayan Griffon Vulture, Indian Burrowing Frog, Ornate narrow-mouthed Frog, Dhaman, Leopard Gecko, etc. A total of 61 plant species were identified in the study area among which dominant species included *Acacia modesta*, *Dalbergia sissoo*, *Olea ferruginea*, *Zizphus nummularia*, *Justacia adhatoda*, *Cynodon dactylon*, *Dodonea viscosa*, *Adiantum incisum* and *Geranium willichianum*. Population of most wildlife species including Punjab Urial has declined over the time. Human-wolf conflict was also noted in Kala Chitta National Park and around 30 domestic animals were killed by wolf during 2017. Major threats to wildlife in the park include illegal hunting, habitat loss / degradation due to livestock grazing, fuel wood / grass cutting. There is an urgent need to protect existing wildlife populations and their habitat in the park. Livestock grazing and removal of natural vegetation by local people for various purposes must be controlled in order to for protect and conserve wildlife habitat. Awareness among general public about importance of wildlife and biodiversity needs to be increased.

POPULATION DENSITY AND HABITAT ASSOCIATION OF HIMALAYAN IBEX (*Capra ibex*) IN QURUMBER NATIONAL PARK, GILGIT-BALTISTAN

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Present study was conducted to determine population density and habitat association of Himalayan Ibex (*Capra ibex*) in Qurumber National Park, Gilgit-Baltistan. It also highlights major threats to Himalayan Ibex in core and buffer zone of the park. Nine study sites were selected in the park to assess distribution and population of Himalayan Ibex where animals were counted using fixed point direct count method. Habitat analysis was carried out using quadrat method in selected sites in its habitat. A total of 474 Ibex were counted during 2015-16, including 132 Females, 90 Males, 132 fawns, 90 yearlings and 31 not determined (N.D) while trophy size males were 23. Total fawns were 27.78% of whole population, followed by yearlings 19%, females 27.79%, males 18.95% and not identified 6.46%. Sex ratio of Himalayan Ibex in the study area was; male and female 1:1.23, female and kid ratio 1:1, female and yearling 4.32:1. Maximum number of Ibex was sighted in

Qurumber sub-catchment; 110 (23%) followed by Borth 82 (17%), Dawjarab/ Bazarkoto 75 (16%), Badswat 74 (16%), Mataramdan 53 (11%), Shamsabad 31 (7%), Bolantar 20 (4%), Thishnalot 16 (3%) and Payakeem nullah contain 13 (3%). Population density of Himalayan Ibex in the study area was 0.63 animals/km² and biomass was 43.7 kg/km². For habitat association of Ibex, vegetation analysis of the study area was carried out by calculating Importance Value Index (IVI) of plants species found in the quadrates. A total 49 plant species were recorded belonging to 28 families. Herbs were dominant flora (51%), followed by shrubs (29%), grasses (8%) and trees (12%). Dominant families were *Asteraceae*, *Betulaceae*, *Pinaceae*, *Cupressaceae* and *Rosaceae*. Out of 49 identified species in park 25 were herbs, 14 were shrubs, 6 were tree and 4 were grasses. Major threats to Himalayan Ibex identified in the buffer zone and core zone of Qurumber National Park based on field observations and questionnaire survey were poaching, habitat fragmentation, deforestation and predation.

**POPULATION DENSITY AND HABITAT ASSOCIATION OF BLACK PARTRIDGE
(FRANCOLINUS FRANCOLINUS) IN KALA CHITTA NATIONAL PARK**

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Present study assessed population density and habitat association of Black Francolin (*Francolinus francolinus*) in Kala Chitta National Park. Three different habitat types were selected in potential habitat of this bird including Mountainous forest and associated grassland, Open cultivated land, and Wetland and associated natural vegetation for collecting data. Data on population was collected along open width transects having length of 1 km in each habitat type. For vegetation analysis 10 quadrates were taken in reach transect. Data recorded on different variables within each habitat was analyzed using ANOVA and Kruskal-Wallis test. Overall population density was 1.06 birds/ha in the study area. population density in Mountainous forest and associated grassland was 2.19 birds/ha, in Open cultivated land and Wetland and associated natural vegetation was 0.84 birds/ha and in Wetland and associated natural vegetation was 0.15 birds/ha. Analysis of data revealed that population densities were significantly different between three habitats ($F=20.82$, $P=5.70$, $d.f=26$) at $\alpha=0.005$. In total, 59 plant species were recorded in habitat of Black Francolin, including 8 trees, 9 shrubs, 35 herbs and 7 grasses. Dominant species were *Olea ferruginea*, *Morus alba*, *Acacia modesta*, *Acacia nilotica*, *Albizia lebbek*, *Dodonaea viscosa*, *Prosopis juliflora*, *Achyranthes aspera* linn, *Ziziphus nummularia*, *Carissa opaca*, *Scirpus maritimus*, *Malvastrum coromande*, *Parthenium hysterophorous*, *Adiantum incisum*, *Cynodon dactylon*, *Saccharum bengalense*, *Aristida funiculata*, *Cenchrus ciliaris* and *Cymbopogon jwarancuse*. Most preferred habitat of Black Francolin in the study area was Mountainous forest and associated grassland having higher population density (2.19 birds/ha) dominated by *Olea ferruginea*, *Dodonaea viscosa*, *Acacia modesta*, *Ziziphus nummularia*, *Morus alba*, *Acacia nilotica*, *Aristida funiculata*, *Parthenium hysterophorous* and *Cynodon dactylon*. Further studies on Black Francolin such as population dynamics, feeding pattern, breeding biology, major threats, etc are strongly suggested.

**POPULATION STATUS AND HABITAT ASSOCIATION OF HIMALAYAN MONAL
(LOPHOPHORUS IMPEJANUS) PALLAS VALLEY, KOHISTAN**

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Present study was conducted with the objective to determine the population density and habitat association of Himalayan Monal Pheasant (*Lophophorus impejanus*) in Pallas valley, district Kohistan, Khyber Pakhtunkhwa. For data collection, three different habitat types were selected in the study area including i) Karosair mixed conifer forest, ii) Deewan Nallah Fir-Spruce and Betula forest and iii) Kabkot Fir-Spruce forest habitat. Each habitat was further sub-divided into 3 - 4 study points. Dawn call count method was employed to record data on population. A total of 21 birds were recorded during the study, which resulted into population density estimation of 7.5 ± 3.2 birds/km² in the study area. Population density of birds recorded in the Karosair

mixed conifer forest habitat was highest (8.9 ± 2.30 birds/km²), followed by Dewan Nallah Fir-spruce and Betula habitat (7.14 ± 2.06 birds/km²) and Kabkot Fir -spruce habitat (5.95 birds/km²). Population density of Monal pheasant not significantly different in three habitat types ($F = 2.31$; $df = 26$; $p\text{-value} = .1208$; at $\alpha=0.05$). For assessment of habitat association, Quadrature method was used and 10 quadrates were taken in each transect / habitat type. A total of 54 plant species recorded from Monal habitat, including 7 trees, 10 shrubs, 37 herbs and grasses. Dominant trees were *Picea smithiana* (IVI= 14.88), *Abies pindrow* (IVI= 13.86), *Betula utilis* (IVI= 11.60), *Pinus Wallichian* (IVI= 10.89), *Quercus incana* (IVI= 5.29) and *Cedrus deodara* (IVI= 4.18). Dominant shrubs were *Viburnum nervosum* (IVI= 22.08), *salicifolia* (IVI= 16.17), *Bebris lyceum* (IVI= 15.06), and *Dehpne oleoides* (IVI= 19.37). Dominant herbs and grasses were *Colachicum luteum* (IVI= 10.56), *Corydalis govaniiana* (IVI= 9.58), *Rumex dentatus* (IVI= 9.32), *Primula denticulatae* (IVI= 9.31), *Oxalis corniculata* (IVI= 8.67) and *Dryopteris juxtaposita* (IVI=8.20). In Pallas valley, this species is hunted during the breeding season when it is easy to locate. Illegal Hunting, egg collection and habitat disturbance are major threat to Monal Pheasant in the study area. Results suggested that a reasonable population of Monal pheasant exists in the study area which needs to be protected for its conservation.

MINERAL CONTENTS IN DIET OF BLACK BUCK (*ANTELOPE CERVICAPRA*) COLLECTED FROM DIFFERENT CAPTIVE AND SEMI-NATURAL CONDITIONS

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Foodstuff from different captive sites Lahore Zoo, Bahawalpur Zoo, Lahore Zoo Safari and Lal Sohanra National Park Bahawalpur was collected to determine the mineral content in diet of Black Buck. Food variety was different at different sites. Animals were feeding on same food i.e. Lucerne and parched grams at three captive sites, Lahore Zoo, Bahawalpur Zoo and Lahore Zoo Safari. In contrast to that animals in Lal Sohanra were feeding on grain, berseem (*Trifolium alexandrinum*), oat (*Avena sativa*), Lucerne, Jowar (*Sorghum bicolor*) Kikar (*Prosopis juliflora*) Beri, Jandi (*Prosopis cineraria*) at regular basis during the survey durations. Foodstuff was analyzed through mineral extraction method to find out the mineral concentration in fodder and diet. All samples were analyzed under standard protocol in Veterinary Research Institute lab Lahore. Minerals such as Na, K, Ca, Mg, and P were observed in various concentrations along with the trace elements. It was noticed that there were striking differences in mineral contents of Black Buck fodder collected from all enclosures. Black bucks of Lal Sohanra were having remarkable body sheen, breeding rate, amazing physical appearance. This was due to their better diet at their enclosure. Natural behavior of these animals was seen only in animals of Lal Sohanra while all these characters were almost absent in black bucks of other sites. We concluded from the study that these threatened animals are being compromised in captive conditions. There is need to revise the adapted ration scale by management of captive sites.

DIET COMPOSITION OF MIGRATORY HOUBARA BUSTARD (*CHLAMYDOTIS UNDULATA MACQUEENII*) IN DISTRICT DERA ISMAIL KHAN

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Houbara Bustard (*Chlamydotis undulata macqueenii*) is a medium-sized bird of the family Otididae which migrates along green route through Indus flyway (4) visiting several plain areas of Pakistan. Those

migrating central and east Kazakhstan winter in Afghanistan, Pakistan and southern Iran. District Dera Ismail Khan in Khyber Pakhtunkhwa lies in its route and provides winter refuge for Houbara species visiting Pakistan. Present study on feeding and habitat association of Houbara bustard was conducted in district Dera Ismail Khan from November 2015 to June 2016. Two study sites were selected for collecting fecal samples and reference plant species including Ganju and Jhok Machi. Fecal samples were collected from Houbara bustard habitat and analyzed micro-histologically. The droppings were identified from their shape, dimension, structure and presence of grain particles and sign of foot prints nearby. In connection with fecal pellets, potential vegetation (Trees, Shrubs, Herbs and Grasses) were collected as reference material for making slides. Results showed that fruit and leaves of *Capparis decidua*, flowers of *Brassica campestris* and a herb *Arva javanica* were most preferred food items. Houbara bustard is an omnivorous bird as ants and beetles were also present in their diet. Habitat analysis was carried out through vegetation survey taking 20 quadrates in both study sites. Twenty-one plant species were recorded from of Houbara bustard habitat out of which 6 were trees, 5 shrubs, 5 herbs, 3 grasses and 2 cereal crops. Dominant plant species were *Capparis decidua*, *Acacia nilotica*, *Zizyphus numelaria*, *Zizyphus oxylpha*, *Salvadora oleoides*, *Prosopis cineraria*, *Pegnum harmala*, *Medicago sativa*, *Brassica campestris*, *Tamarix aphylla*, *Aerva javanica*, *Bassia indica*, *Cynodon dactylon*, *Symbopogan schoenanthus* and *Desmockya bipannaata*. Preferred habitat of Houbara bustard is open areas with sparse vegetation and it mostly like shades of *Capparis decidua* and hides underneath. Hunting and habitat degradation should be strictly controlled for its conservation in the study area.

FIVE TO SEVEN KINGDOM SYSTEMS OF ORGANISMS IN BIOLOGICAL SCIENCES

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Robert Whittaker proposed two to five kingdom system scheme in 1969. The modification in Whittaker's scheme were made by L. Margulis and K. Schwartz in 1985 as Kingdom Prokaryotae (Monera): It includes all prokaryotes eg bacteria and cyanobacteria. Kingdom Protocista (Protista): It includes all unicellular eukaryotic organisms, which are no longer classified as fungi, plants and animals. Kingdom Fungi: It includes all non-chlorophyllous, multicellular having chitinous cell wall, coenocytic body called mycelium, absorptive heterotrophs. E.g. Agaricus (mushroom) yeast but except budding yeast. Kingdom Plantae: It includes all the eukaryotic, multicellular chlorophyllous photosynthetic autotrophs, having cell wall made up of primarily of cellulose, zygote retained to become embryo and exhibiting heteromorphic alternation of generation e.g moss, fern, pine, mango etc. Kingdom Animalia: It includes all eukaryotic non-chlorophyllous, multicellular, ingestive heterotrophs, having no cell wall e.g Hydra, Earthworm, fishes, Amphibians, Reptiles, Birds, Mammals including Monkeys, Apes and Humans. Methods Used Literature search method was used, for these different books and journals were studied. In the above current 5 kingdom system prions, viroids and viruses are not included, the reason centers on the controversy, which has been going on ever since they were discovered, as to whether or not they should be regarded as living. A prion simply consist of protein, a viroid simply consist of Nucleic acid and a virus simply consist of nucleic acid surrounded by a protein coat, and they can only survive and reproduce inside a living cell. For these reasons most biologists regard them, not as living organisms, but as aggregation of molecules similar to those normally found in living cells. Prions, Viroid and Viruses appear to be on the borderline between the living and non-living worlds. They could probably form another kingdom. Which is being proposed as: Kingdom Akaryotae (Endocytia) Akaryotes, only protein present e.g prions or only nucleic acid (RNA or DNA) present e.g viroids or both protein and nucleic acid present, when both protein and nucleic acid are present the protein coat covers the nucleic acid e.g viruses, they survive and reproduce only inside the living cell, have both characteristics of living (when inside the living cell) and non living (when outside the living cell), due to both living and non-living characteristics, they may be assumed as connecting link between living and non-living worlds. eg. Prions, viroids, viruses. Kingdom Humania: Eukaryotic multicellular, non-chlorophyllous presence of chin & lips, have reduced hairs on body, have speaking, smiling, laughing, writing, reading and inventing power. Make use of fire for different purposes. Prions, viroids and viruses were not included in five kingdom systems, they may be assumed as connecting link between living and non living worlds. Animals and humans are two different things, the mixing of these two kingdoms is likewise the mixing

of fungi and plants, separated on one point. Presence and absence of Chlorophyll.

Seven kingdoms may be listed as below:

1. Kingdom Akaryotae (Endocytia) 2. Kingdom Prokaryotae (Monera) 3. Kingdom Protocista (Protista) 4. Kingdom Fungi 5. Kingdom Plantae 6. Kingdom Animalia 7. Kingdom Humania.

ECOSYSTEM SERVICES OF RODENTS IN AGRICULTURE: A REVIEW

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The concept of indicator species has also been adopted for rodent's species as a measure of the health of an agricultural landscape. The high diversity of rodent species in the agro-ecosystems may provide an opportunity to identify species that can indicate whether the ecosystem is in poor condition (degraded landscape) or in good condition (sustainable production is likely). A review revealed that rodents are prolific breeders and often represent a significant amount of the animal biomass in forests and other natural ecosystems. They are also important as environmental and soil engineers, helping to spread pollen and seed, aerating the soil through their digging and burrowing activities. These ecological benefits are called 'ecosystem services'. Indian crested porcupine (*Hystrix indica*) is largest rodent found in Pakistan, it has aesthetic importance and its meat is also believed to have a medicinal value for arthritis patients. Among small mammals, the most thoroughly studied hoarder behavior is the lesser bandicoot rat (*Bandicota bengalensis*) in the croplands of Pothwar area. They construct extensive burrow systems and accumulate food in under-ground chambers, scattered surface caches, or both which may provide food to many invertebrates. Our review suggests that rodent's species play particularly large roles in agro-ecosystems and create an environment that would change dramatically if they disappeared. As such, they play an important role in the food web, both as consumers of plants and fungi. Much could be learned from rodents that may be better indicators of agricultural health at a local to regional scale which could further clarify and expand our knowledge of rodent species.

BIODIVERSITY AND ECOLOGY OF ANATIDAE FAMILY OF ANSERIFORMES INHABIT AT MANCHAR LAKE SINDH, PAKISTAN

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On the basis of biodiversity of the various genera of captive Family of Anatidae order anseriformes inhabit at Manchar lake Sindh, Pakistan were carried out from 10th February 2017 to 16th August 2017. The total number of 30 specimens consisting number of 13 males and 17 females were studied taxonomically and morphologically as well. The study of total number of 03 species of family Anatidae order anseriformes including genus '*Anas*' (Green winged teal), '*Anas platyrhynchos*' (Mallard Male), and '*Anas platyrhynchos*' (Mallard Female) were studied and identified with various international parameters. On the basis of ecology of the Manchar Lake the Physico-chemical parameters were carried out at monthly intervals. A pH meter was used for the analysis of hydrogen ion concentration, whereas conductivity meter was used to record the value of electrical conductivity (EC) and total dissolved solids (TDS). The concentrations of Total hardness (Calcium-Magnesium) were determined by titration procedure and the Salinity, Turbidity, Sulphite, Chloride, Nitrate, Arsenic etc were also determined. The captured species were measured with various parameters as under:

- (i) Body length from tip of beak to cloaca (ii) Body weight (iii) Body color dorsal and ventral (iv) Length of tail from cloaca to the tip of tail. (v) Length of fore limb (vi) Length of hind limb (vii) Measurement of feet and web. (viii) Color of wings and eyes These parameters have measured with different variation of body as well as colors.

ASSESSMENT OF CROPS DAMAGE CAUSED BY INDIAN CRESTED PORCUPINE (*HYSTRIX INDICA*) BY FOOD HABIT ANALYSIS IN DISTRICT BAGH AZAD KASHMIR

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The Indian crested porcupine (*Hystrix indica*) is the second largest rodent pest species in the world. It causes huge economic loss as it feed upon agricultural lands and fruit trees. The main crops damage by porcupine is maize, wheat, potato, melon, pumpkin, chilies, tomato and sugar cane. The other main non cultivated plants are *Pinus roxburghii*, *Dilbergia sissoo* and *Eucalyptus* species. Unfortunately, the problem was neglected in AJK and no formal data is available regarding it. Therefore, the present study has been conducted to assess the magnitude of damages caused by the species in district Bagh. The crop damage assessment was carried out by direct visualizations, fecal count and stomach analysis. The data collected by direct visualization from ten study sites showed the maximum relative percent damage for potato (40.15%) followed by maize (39.97%) and wheat (10.07%). Potato was highly damaged than maize ($p=0.001$) and wheat ($p=0.001$). While the damage to maize was significantly higher than wheat ($p=0.001$). The analysis of fecal and stomach confirmed thirty one plants species from fecal pellets and twenty seven from stomach. The data showed that crops (23.73%), vegetables (9.35%), herbs (18.84%), shrubs (0.34%) grasses (7.52%) and trees (3.66%) were consumed by Indian crested porcupine. Among plant species *Zea mays*, *Diopyrus lotus*, *Melia azedarach*, *Rumex obtusifolius* and *Pinus wallichiana* were highly preferred. The seasonal variation among crops showed that *Brassica campestris* was preferred in winter while *Zea mays* in fall. On the basis of the findings, conservation strategy must be proposed to minimize the problem in the area.

EXPLORATION OF GENERAL FAUNA OF KALA BAN JANGLE IN DISTRICT BAGH AZAD JAMMU AND KASHMIR

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The current study has been conducted to explore the general fauna of Kala ban Jungle district Bagh (AJ&K) from May 2016 to December 2016. For this purpose direct method (field visualization) and indirect methods (hunting/ killing record and questionnaire survey) were used. A total of 32 (N=32) species belonging to four classes (mammals, birds, reptiles and amphibians) were recorded over there. The mammals (n=11) include Common leopard, Wild boar and Indian civets). Among birds (n=8) the pheasants (Koklas, Monal and Khali) were noticed to be existed in the area. Moreover, reptiles (n=10) include Kashmir Rock Agama, Mediterranean gecko, Fat tail gecko and western three-lined skink and three species (n=3) of amphibians were sorted out. The current study shows presence of magnificent wildlife of the area that should be further explored.

EXPLORATION OF SMALL CATS, THE ASSOCIATED THREATS AND CONSERVATION MEASURES IN NORTHERN SIX DISTRICTS OF AZAD JAMMU AND KASHMIR

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Contributing a great deal in food chain, aesthetic aspect and rodent's control, small cats have a great importance in the ecosystem. Despite, there is no published literature available on existing cat species in Azad

Jammu and Kashmir (AJ&K). The current research work (August 2016-October 2017) was designed to explore the small cats in AJ&K and to establish an inventory of the species existed in the area. For this purpose, the direct visualization of the species (field photographs) and secondary data collection methods (questionnaire surveys) were employed. A total of (N=361) individuals including (wild cats n=43 and feral cats n=318) were directly visualized in the natural habitat. The wildcats that were visualized directly included Leopard cat (n=10), Fishing cat (n=07), Marbled cat (n=05), Jungle cat (n=17), Asiatic golden cat (n=02) and Lynx (n=02). There was no significant difference for presence of feral and wild cats among the districts while observed directly. The presence confirmed by respondents was found significantly different ($p<0.005$) among the studied districts. The harmful impacts posed by both species (cats and humans) on each other were also assessed through interviews of local community in the study area. A significant difference was recorded for the losses due to cats among the six studied districts as they killed hens and chicks (44.86%, $p=0.000$) and Pigeons (31.96%, $p=0.000$). They were also found responsible for the household losses (23.18%, $p=0.000$) consequently, they were being killed by human in revenge. The methods of killing for small cats were also interrogated and shown a significant difference among all the districts. The methods used for killing of cats, incorporated: Killing by Guns (16.44%, $p=0.001$), killing by means of dogs (28.81%, $p=0.000$), Use of common approaches (Stone, sticks) (12.04%, $p=0.000$), Accidental killing (24.97%, $p=0.000$) and poisoning (17.74%, $p=0.001$). The respondents were also asked, whether the cats should be protected (39.05%) or not (60.95%) and also shown a significant difference ($p=0.000$) of opinion among the studied districts. The field data was analyzed by Chi-Square and ANOVA by using SPSS version 16.00. The area was found rich for feral cats and it needed no any conservation plans while wild cats were found less in number indicating, that they need subsequent studies for further confirmation of actual threats to wildcats.

**FOOD HABITS, FEEDING SEASONALITY AND ASSESSMENT OF DAMAGE INFLICTED BY THE
SMALL KASHMIR FLYING SQUIRREL (*HYLOPETES FIMBRIATUS*) IN
DISTRICT BAGH, AZAD JAMMU AND KASHMIR**

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The Small Kashmir Flying Squirrel (*Hylopetes fimbriatus*) is a frugivore rodent, hence causes substantial economic loss. The current study highlights the harmful effects of flying squirrel on fruit trees in district Bagh, Azad Kashmir during a period stretching from November 2016 to November 2017. The study area was divided into ten study sites and forty-thousand-meter square area was visited. The damage was assessed by counting numbers of affected trees (wild and planted). The stomach content and fecal pellets were also analyzed to find the food habits of the species. The results of direct visualization revealed that the squirrel species causes 51.90% fruit loss from fifteen different fruits species. In Summer, Winter and Fall cultivated fruits while during Spring season wild plants were deteriorated. During Summer (44.85%), Winter (31.38%), Fall (37.53%) and Spring (83.21%) fruits were ruined by the species. From collected four stomachs samples of Fall Season, the parts of eight (n=08) plants species were obtained. Highest Relative Frequency was found of *Pyrus communis* (49.32 %). Only three plants species, some unidentified material and Leaves were recovered from stomach content during Spring. *Quercus incana* (30.28%) have maximum Relative Frequency. In Summer season, the highest Relative Frequency was found for *Juglans regia* (44.33%). Similarly, in Winter Uppermost Relative Frequency was recorded for *Pyrus communis* (49.32%). The food habits of species were also analyzed through fecal pellet analysis. A total of four hundred fecal pellets (ten pellets from each study sites so hundred in each season) were analyzed in different seasons. The fragments of five fruits species were recovered from Winter scats. The highest fragments were found of *Prunus padis* (26%). From Spring scats only three Wild plants were obtained and the maximum particles were found of *Pinus wallichiana* (46.67%). In Summer scats, parts of six fruits were gathered. Most of the particles were belongs to *Juglans regia* (32.94%) and lowest belongs to *Prunus domestica* (2.35%). Whereas the scats of Fall contain fragments of eight different fruits species. Highest parts were of *Juglans regia* (22.13%). The morphometrical analysis (length, width and weight) of scats were

also carried out to identify the seasonal effects of food items. The largest scats size was found in Summer while smallest size was recorded in Fall season. Similarly, the widest fecal pellets were also measured in Summer and least width were found in Fall season. The highest weight of scats was found in Summer and lowest were found in Spring season. The area needs further exploration.

GROWTH RATE OF (*POMACEA CANALICULATA*) APPLE SNAIL AGAINST FEEDING FREQUENCY

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The freshwater Apple snail *Pomacea canaliculata* is a prominent paddy field pest in Southeast Asia. The present experiments are part of a larger study designed to investigate the influence of feeding frequency on the growth of the apple snail. The laboratory experiments revealed that high frequency feed has great influence over low frequency feed on growth of these animals. Among various physico-chemical variables such as Dissolved Oxygen, conductivity, salinity, temperature and pH recorded during present study, the growth and feeding rates were found to be mainly effected by temperature. In this study the effect of feeding on juvenile and adult growth were focused. The two adult snails and four juvenile were stocked in two separate aquaria. Snails were fed at two frequencies, half of which were fed four times/day feed and another half were fed two times/day feed during five months of the experiments. The experiments were conducted under a 12 h light: 12 h dark photoperiod. Growth parameters of snails were evaluated after two weeks of administration. The feed consumption and physio-chemical of the environment were observed. During the feeding test the difference in food consumption among adult and juvenile snail was noted that juvenile fed more than adult snails. It was also observed that the group of snails was achieved more weight and length which were fed two times in a day than the group which were fed four times per day. The rapid growth was observed in May. The favorable temperature range for the growth of *P. canaliculata* was found to be 28°C-31°C.

A STUDY ON THE MORPHOLOGICAL FEATURES OF DOLPHIN SKULL *STENELLA* SPP. (GRAY, 1866) (FAMILY: DELPHINIDAE) FROM THE COAST OF PAKISTAN

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Cetaceans are diversified group of mammals distributed throughout the world. It consists of two suborders Odontoceti and Mysticeti with 41 genera and 79 species. One of the diverse families of the suborder odontoceti is family Delphinidae. This is the largest family with 32 species and 17 genera. Marine mammals are reported to occur frequently in Pakistani coastal and offshore waters. The knowledge on cetaceans in Pakistan has improved immensely. This study deals with detailed morphological description of genus *Stenella* (Gray, 1866) (spotted dolphins) with five species that are found in tropical and temperate seas of the world. The skulls of *Stenella* spp. were collected from the Sindh (89 Km) and Balochistan (96 km) coasts. The detailed study was done to identify dolphin species on the basis of morphological parameters. This study will contribute in the baseline biological information that will be helpful in facilitating the development of management strategies in relation to the conservation of cetacean species in Pakistani waters.

MOLECULAR BIOLOGY SUPPORT FOR BIORESOURCE MANAGEMENT**Zahid Iqbal Khan***Bioresource Research Centre (BRC), 34-Bazaar Road, G-6/4, Islamabad*

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Bioresource is any nonfossil resource having a biogenic origin, which can be used by human for multitude of purposes. However, leaving bioresource un-harvested is the loss of resources for the period it remained unexploited, while overharvesting results in complete loss of the resource for ever. Therefore, Bioresource management was not a serious issue, when the human population was small and its demand for the bioresources was limited. Maximum harvest of the bioresources remained much below total regeneration potentials and thence the population level of bioresource species was maintained at the carrying capacity of the area under ecological feedbacks. Unfortunately, due to increase in population there is continuous exploitation of bioresources due to which there is dire need for Management of bioresources within balanced harvest approach. The management policy of a balanced exploitation need to be based upon reliable information on: 1). Proper identification of resource, 2). Assessment of present resource potentials and monitoring changes over the time, and 3). Controlling illegal harvesting. Collection of reliable information on all these aspects was difficult and time consuming before the advent of the molecular biology techniques. Thus, current presentation tries to give an overview of the conventionally and modern molecular biology based techniques available for collecting such information.

WHAT IS IN MENU FOR COMMON LEOPARD IN NORTH-EASTERN HIMALAYAN REGION OF PAKISTAN?**Faraz Akrim^{1*}, Tariq Mahmood², Muhammad Sajid Nadeem³ and Shaista Andleeb⁴**^{1,2,4}*Department of Wildlife Management, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi*¹*Department of Zoology, University of Kotli, Azad Kashmir*³*Department of Zoology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi*

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Knowledge of a predator's diet is important for understanding its ecology and for predicting its influence on the dynamics of prey populations. We investigated diet composition of common leopard at Pir Lasura national park, Azad Jammu and Kashmir, Pakistan. We used molecular identification technique to identify scats of common leopard. Prey species of common leopard were identified using prey remains in scats. We identified scats of common leopard with 96% accuracy. Diet of common leopard comprised of 17 prey species (10 wild and 7 domestic). Frequency of occurrence of wild prey was 34.85 % of total leopard diet whereas domestic prey contributed 59.1%. Results of current study showed that common leopard is mainly subsisting on domestic animals which may result in negative human-carnivore interactions. We suggest that local communities should be educated to save leopard and its prey also incentives should be introduced for local communities to increase acceptance for common leopard in their surroundings.

SECTION – V I

POSTER SESSION

**THE STUDY OF VARIOUS EXTRACTS OF GREEN PLANTS AS BIOPESTICIDES TO
CONTROL THE LEAFHOPPERS (*AMRSCA SPP*) POPULATION ON
THE AUBERGINE (*SOLANUM MELONGENA L.*)**

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The experiment was conducted to know about difference between the bio-pesticide against aubergine jassid. The studies were performed at Institute of Agricultural sciences, University of the Punjab Lahore. Randomize Complete Block Design (RCBD) was used with the four replication. The aubergine genotype (bemisaal) was selected as host plant resistance and following biopesticide were used to control the leafhopper i.e Garlic extracts + surfactant (700ml+200gm/acre), Neem leaf extracts + surfactant (600ml+100gm/acre), Tobacco leaf extracts +surfactant (600ml+100gm/acre), Ginger extracts +Surfactant (500ml+100gm/acre), moringa leaves extracts and orange leaves extracts (500ml). The data was collected after 24 hours, 48 hours, 72 hours and 7 days of application. The neem extract + surfactant showed the very effective leafhopper control and its mortality was 69.52% while orange leaves showed the minimum control of leafhopper and its mortality was 27.87%. The garlic, tobacco and Ginger extracts+surfactant showed following mortality 57.45%, 55.43% and 45.89% respectively. The significant differences were noted in the yield of each treatment. The MSTAT was used for data analysis with the LSD >.05

**OCCURRENCE OF *BACTEROCERA* SPECIES AS A MAJOR PEST OF GUAVA
FRUIT ORCHARDS AT LARKANA DISTRICT, SINDH**

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In Sindh Province Larkana division is largest Guava grower, Area comprises of 3062 hect: with production of 23371 M.tons. Different varieties of guava are grown and export to other cities as well as to foreign countries. Fruit flies *bactrocera* (Diptera: Tephridae) are amongst the most important pests attacking fruits worldwide and usually attack commercial fruits guava, peach & oranges and citrus fruits. In Larkana Guava fruiting 2 times in a year: 1st season start from 15th October to 15th of April 2017, 2nd season for fruiting starts from 5th June to August 2017 (The guava produced from 15th October to 15th April are locally consumed as well are exported to other areas. While the 2nd seasonal guavas 5th June to August ; are entirely consumed locally. because of sever hot weather and less shelf life. It is also pertinent to say that the 2nd produce is very much destroyed by fruit fly attack. The most common fruit fly species of genus *Dorsalis* were trapped, through methyl eugenol traps identified and preserved through this research study, rate of damage/loss 60- 80% reported during the month of June and August 2017, and 20-30% loss reported during 15th October to 15th of April 2017. The result suggest that temperature effects the rate of infestation of fruit fly genus *Dorsalis* at guava orchards of Larkana.

BIODIVERSITY OF EARTHWORM SPECIES FROM VARIOUS HABITATS OF DISTRICT FAISALABAD, PAKISTAN**Iqra Arif and Abdul Ghafoor***Department of Zoology University of Sargodha Women Campus, Faisalabad*

The present research was planned to find out the population distribution of earthworms from crop lands, gardens grassy fields and water channels from different localities of Faisalabad. Like Gutwala, Ayub research agriculture institute Jhang road; Smundari road Faisalabad. 11 species of earthworms i.e. *pheretima diffringens*, *pheretima hawayana*, *pheretima elongeta*, *pheretima morrisi*, *Drawida nepalensis*, *Eutyphoeus pheretima incommodus*, *Eutyphoeus waltoni*, *Lampito mauritii*, *Dichogaster bolau* and *Dichogaster modigliani*, belonging to five genera, *Pheretima*, *Drawida*, *Eutyphoeus*, *Lampito* and *Dichogaster* and three families, *Megascolecidae*, *Octochaetidae* and *Monilidastridae* were found from the study sites. Maximum number of earthworms were recorded in the month of April, May and October and population dropped in winter (December and January). These findings exhibited great fluctuations in earthworm population over the months and warrant more research on their role to improve the soil for agriculture in the study area and elsewhere in Pakistan.

GENOTOXIC EFFECT OF BINARY METAL MIXTURE IN PERIPHERAL BLOOD ERYTHROCYTES OF COMMERCIALY IMPORTANT FISH, *CYPRINUS CARPIO***Faiza Ambreen¹, Muhammad Javed², Safina Kousar¹, Sidra Abbas², Rahila Ilyas¹, and Fariha Latif²**¹*Department of Zoology, GC Women University Faisalabad, Pakistan*²*Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan*

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The present experiment was undertaken to determine the genotoxic effect of binary metal mixture of cadmium and lead in peripheral blood erythrocytes of commercially important fish, *Cyprinus carpio* (common carp) by using Single Cell Gel Electrophoresis (SCGE). In 1st step the 96-hr LC₅₀ value of mixture was determined for this fish in a static system and then four sub-lethal concentrations viz. SL-I (10.42mgL⁻¹), SL-II (7.81mgL⁻¹), SL-III (6.25mgL⁻¹) and SL-IV (5.21mgL⁻¹) were calculated. Fish were exposed to these concentrations, separately in glass aquaria for 30 days along with negative and positive control at constant laboratory conditions. Peripheral erythrocytes were sampled after 30 days of exposure for genotoxicity assessment. Statistically significant (p<0.05) effects were observed at sub-lethal concentrations in-terms of percentage of DNA damage, cumulative tail lengths (µm) and genetic damage index. Concentration dependent response was observed in fish erythrocytes with induction of maximum DNA damage, due to positive control, followed by at highest concentration (SL-I) of mixture. This study also concluded that SCGE can be used for in-vivo experiments, using fish as a model for the screening of genotoxic and mutagenic pollutants in aquatic environment.

CARDIOVASCULAR RISK ASSESSMENT IN HIGH ALTITUDE RESIDENTS**Hafiza Saba Yasin, Sosan Andleeb Khan and Nabila Roohi**¹*Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore- 54590*

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At 3658m, the barometric pressure falls and there are 40% fewer oxygen molecules per breath, thus lowering oxygen supply to the body tissues leading to hypoxia. Residents at high altitude have an active life style so their lipid profile is different than that of the sea level inhabitants. This study determines the cardiovascular risk assessment at high altitude by studying their lipid profile and comparing it with that of the Punjab plain residents. Blood samples of 30 healthy high altitude residents were selected from Rawalakot (5300 ft), AJK and 30 Punjab plain residents from Lahore (712 ft). Lipid profile includes quantitative determination of cholesterol and triglycerides of the residents of high altitude and Punjab plains by using clinical chemistry

analyzer (Model 5010, Robert Riele GmbH & Co KG, D-13467 Berlin, Germany). While LDLs were calculated by using formula TG/5. Intergroup comparison was made to access the variations. Cholesterol showed significant decrease in inhabitants of high altitude when compared with Punjab plain residents. There was non significant increase in triglycerides and LDLs at high altitude as compared to residents of plain level. It may be concluded that people living at high altitude have low cardiovascular risks than that of plain level.

HEDGEHOGS *P. MIRCOPUS* AND *P. HYPOMELAS* JERDONI SECURE HUMAN AGRICULTURAL INTEREST (RICE, SUGARCANE AND VEGETABLES) FROM DANGEROUS INSECTS AND OTHER ANIMALS OF THE DISTRICT KAMBAR-SHADADKOT SINDH-PAKISTAN

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During present study, from March 2016 to August 2016, 75 specimens of Hedgehogs were randomly recorded from different localities of District Kambar-Shadadkot. In these areas, different agricultural crops such as rice, sugarcane, wheat, cotton and vegetables are cultivated. Hedgehogs are very popular and attractive group of mammals, which are active at nights, and provide aeration to the roots of the pastures without damaging them by making deep burrows. Hedgehogs being insectivorous insure great economical importance so for the agricultural crops are concerned and due to their feeding habits they help in cleaning crops from different type of dangerous insects and other animals. As the result (75) individuals of 02 species viz; *Paraechinus mircopus* and *Paraechinus hypomelas Jerdoni* to Sub Genus *Paraechinus*. Out of 75 specimens, 50 belong to *P.mircopus* and 25 specimens of *P.hypomelas Jerdoni*. Wherein *P.mircopus* show raised population in comparison to *P.hypomelas Jerdoni* which in human agricultural interests.

ECOLOGY AND OCCURRENCE OF *BAGARIUS BAGARIUS* (HAMILTON, SISORIDAE; SILURIFORMES) FROM INDUS RIVER JAMSHORO SINDH PAKISTAN

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Jamshoro is located on the west bank of River Indus. It is about 220 kilometers from north to south and about 100 kilometers wide from east to west. About 2 to 6 kilometers wide belt of the west bank of River Indus is cultivated and irrigated and the remaining land of the district is either mountainous or cultivated. The River Indus is the most important supplier of water resources to the Sindh and Punjab plains; it forms the backbone of agriculture and food production in Pakistan. The river is especially critical since rainfall is meager in the lower Indus valley. The bony fishes are the most common found worldwide. *Bagarius bagarius*, fish also found in the bottom of Indus River. *B. bagarius* lives fast and rocky pools of large and medium-sized rivers. They breed in the rivers prior to the starting of the yearly flood season. Most of the *Bagarius* species are marketed fresh, and are important as a food fish, but the meat spoils rapidly and can cause illness. They vary from small to large in size and also known as the devil catfish, dwarf goonch or goonch in common Sindhi language called Foji khago. It is a species of catfish in the order siluriformes, genus *Bagarius* and family sisoridae and carnivores in nature they feed on small fishes, frogs and shrimps and entomophagous. The present study was carried out from Indus River Jamshoro Sindh Pakistan from May to September 2017. It plays vital role in Pakistan economy too. Total 253 specimens were collected from study site, with the help of fish catching net and other resources and more prevalent were recorded from study site.

**WEB BUILDING BEHAVIOUR OF *ERIOVIXIA EXCELSA* (ARANEAE: ARANEIDAE)
FROM BHALWAL, PAKISTAN**

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The present study was focused on observing web building behavior of *Eriovixia excelsa*. The specimens were collected from 10 chak NB Bhalwal, Pakistan. These species of spiders are present in citrus fields. There are different parameters of web which include no. of spirals, no. of radii, web diameter, and mesh height around and away from the hub. The web building time is also noted for these spiders. The data of all parameters were recorded, after that all 100 samples which were collected in bottles, were transferred into laboratory. The wet weight and 4 leg length of all spiders were calculated by laboratory equipment's. Then we applied statistical analysis on data which was recorded during research in laboratory. IV leg length and wet weight show variations with all other parameters. The correlation of IV leg length and wet weight was checked with all variables of web, it shows different type of relations. IV leg length and wet weight are crucial parameters for building web. The spiders which has large IV leg length and wet weight means larger spiders build larger web and spiders which having smaller IV leg length build smaller web. So IV leg length contributes a lot for building web. The size of *Eriovixia excelsa* is influenced by several factors like temperature, humidity, precipitation and annual rainfall. These monthly variation of six months were checked on the structure of web, and their P-value was calculated which shows that monthly variation effect the weight of spider and no. of radii in the web.

GENETIC VARIATION OF WILD AND HATCHERY REARED ROHU (*Labeo rohita*)

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Genetic variation at the population level can provide an idea about different genetic status, the genetic diversity among them and their evolutionary relationship with wild relatives. It has been postulated that over successive generations, low effective population sizes lead to inbreeding depression of unplanned hybridization with reduced growth rates, loss of genetic diversity, fecundity and poor survival. The performance of the hatchery-produced fry has been claimed to be declined significantly compared with the natural fry. The present study has been designed for assessment of genetic level of Rohu populations. A total of 35 (mean, 4.38 ± 0.78) RAPD bands were amplified by applying of Eight primers. Overall polymorphism (%) is varied from higher (71.68%) with different level in all RAPD loci and varied among all populations with low (51.12%) of River Chenab. This was ascribed with the significant level of polymorphic information content. Estimates mean values of genetic diversity ($h = 0.27 \pm 0.11$) and Shannon's diversity index ($I = 0.47 \pm 0.17$) for Chenab river populations of Rohu fish were comparatively high varied from high (Ravi, 0.26 ± 0.03) to low (Manawan, 0.16 ± 0.03). Low level of genetic flow (mean, 2.7990 ± 1.0881) among population was also determined that showed the higher level of genetic variation among different populations of hatcheries/farms to natural rivers of *Labeo rohita*. Range of Nei genetic distances (0.0008-0.1477) based on UPGMA model Rohu populations clustered in three main groups corresponding of divergence level. However, results to low level of gene flow exhibited increasing of genetic variation among populations. Decline of genetic diversity (level < 0.5) and significant higher genetic differentiation ($F_{st} = 0.453$; $P \leq 0.001$) revealed the high level of inbreeding coefficient within populations of hatcheries and farms ultimately that effecting the growth size of *Labeo rohita* in natural habitat.

CHEMOTAXIS OF ORIENTAL FRUIT FLY AGAINST METHYL EUGENOL IN PRESENCE OF DIFFERENT FOOD ESSENCES

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Oriental fruit fly, *Bactrocera dorsalis*, is a major and the most destructive pest of mangoes throughout the world. A number of control measures including chemical, mechanical, physical and biological are under practice for controlling this pest. Among these, Male Annihilation technique (MAT) provides best eco-friendly results. In MAT, methyl eugenol (ME) is used as male attractant which decrease their population to restrict the mating with female to reduce the chances of fertility. Concurrently, ME was used in presence of four food essences to enhance the efficacy of methyl eugenol. The experiment was carried out at Bagh e Latif (Mango Orchard) Tando Jam, Sindh in *Randomized Complete Block design* (RCBD). There were five treatments and each treatment had four replications. Twenty Steiner traps were used in the experiment. Treatment 1 (T1) was control having ME 4ML (methyl eugenol 85% + sugar 10% + insecticide 5%). The remaining four treatments have two wicks of cotton; one with same chemicals as in control and other had 4 ml impregnated wicks of different flavours of food essences. Treatment 2 (T2): ME + Lemon essence, Treatment 3 (T3): ME + Pineapple essence, Treatment 4 (T4): ME + Almond essence, Treatment 5 (T5): ME + Bush essence. The results of the study revealed that the highest mean population (947.80±5.81) was trapped in June whereas; the lowest trapped in the month of July (537.04±6.65). T3 captured highest mean population (1782.75±16.41) followed by T2 (1568.00±15.66), T1 (1372.76±12.9), T5 (1370.83±14.57) and T4 (1347.62±17.50) respectively. Results showed that T3 was highly significant ($p < 0.0001$) among all treatments. All most, all treatment correlated positively with temperature except T4 (-0.1854). Negative correlation was observed against humidity in all. It can be concluded that T3 (ME + pineapple essence) delivered best results in trapping oriental fruit flies.

EFFECT OF POLLUTED WATER ON DNA INTEGRITY OF LABEO ROHITA INHABITED IN RIVER RAVI

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Aquatic pollution is responsible for the damage of DNA in different aquatic organisms and fishes. It can cause malignancies, reduced growth, and abnormal development, decreased survival of embryos, larvae, and adults. Genetic analysis of animal relies on high yields of pure DNA and consequent analysis relies on the quantity and quality of DNA. In the present study two different groups reared and natural of fish *Labeo rohita* have been evaluated for DNA damage. Organic method is being used for DNA isolation from different organs. DNA damage was assessed by using PCR and comet assay methods. The mean total fish group weight and length of reared (22.445±0.342g, 12.8±0.12cm) and Natural (20.128±0.483g, 13.55±0.09cm). The quality and quantity of isolated DNA from different organs were observed as tissue>fins>scales of fish *Labeo rohita*. The quality of isolated DNA from reared Rohu in tissue (1.5754±0.5021), fins (1.432±0.023) and scales (1.3453±0.0291) that were found to be no significant different ($P < 0.05$) within reared group and significant different ($P < 0.05$) from natural group that revealed DNA damage in River fish. In other way of estimation, mean DNA quantity (1351.3±43.46) of reared fish showed non significantly ($P < 0.05$) different from natural fish of Ravi populations. There was no significant difference found in both groups. DNA fragmentation was assessed and extracted DNA of reared group fish showed its integrity by showing a single band while natural

samples were showed more than one bands as a fragmented. DNA damage was also checked by comet assay and results were compared in which reared sample and natural samples. In reared group tail was not formed while in natural group tail was formed which show DNA damage in natural group samples of *Labeo rohita* that is the indication of water pollution. This DNA analysis will be suitable and helpful using a reference for as pollution detector for endangered fish species in future studies.

EVALUATION OF EFFECTIVENESS AND IMPACT OF BOILING ON PROPERTIES OF WATER

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Safe drinking water provision is a big challenge of today. Usually people apply disinfection practices at household level. This study was conducted in Rabwah town where people use drinking water mostly after boiling. Various perceptions are related to boiling water. In current study, effectiveness of boiling as well as its influence on physiochemical properties was analyzed. Water samples having turbidity (2 – 13NTU) of Gram positive and Gram negative bacteria were prepared. These samples were boiled for 5, 10, 15 and 20 minutes, and then bacterial growth along with physiochemical properties (before boiling, after boiling, filtration after boiling) was analyzed. Results showed that boiling inhibited the Bacterial growth. Slight increase in pH, Electrical Conductivity (EC), Total Hardness and Magnesium was perceived. Water with initial physiochemical parameters within WHO (World Health Organization) standards remain near range after boiling. Boiling can be a useful practice for the elimination of bacteriological contamination.

IMPACT OF ENDOCRINE DISRUPTORS AND REPRODUCTIVE OUTCOMES IN WOMEN

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Occurrence of miscarriages due to persistent organic pollutants in the world are increasing day by day and it is a serious health issue for women. Many epidemiological researches show the basic connection of persistent organic pollutants with miscarriages. In 2017 a research was performed and the cohort consist of 100 females miscarriages volunteers from Karachi city of Pakistan. For this research blood serum samples were collected from different areas, laboratories, hospitals of Karachi city. For the determination of organochlorine pesticides and polychlorinated biphenyls residues used liquid-liquid extraction method. The association of serum level of POPs and miscarriages calculated through basic statistics (mean, variance, standard deviation, standard error) on IBM SPSS version 22. Concentrations of selected PCBs and OCs were measured by Gas chromatography and were reported in mg/kg. The exposure of endocrine disruptors (PCBs and OCs) may be associated with miscarriages. Our result shows higher mean value (0.356 mg/kg) of pesticides in middle age group (26-32) than the other such as 19-25 younger and 33-39 older age groups. Current study shows the connection between the concentration of pesticides and miscarriages. The mean value shows that the level of pesticides is higher in those women who have primary miscarriage (0.311 mg/kg) history as compared to the other women with the history of secondary miscarriage (0.222 mg/kg) and more than two miscarriages (0.240 mg/kg). This research shows quantitative evidence that support the exposure of organochlorine and polychlorinated biphenyls may associated with the higher risk of miscarriages in the women of Karachi population other covariates such as age, BMI, blood pressure may also associated with this. Present data shows significant bioaccumulation of pesticides in women. Deposition of organochlorine compounds in women show following trend: One miscarriage group > More than two miscarriages group > Two miscarriage group. This study shows the presence of pesticides in environment which accumulate in the adipose tissues by different

ways such as diet, domestic insect killer spray, residential site etc. Presence of these toxins in biological sample shows that these chemicals are still in common practice.

PREDICTION OF SEQUENCE ANALYSIS, SECONDARY STRUCTURE AND 3D-HOMOLOGY MOLECULAR MODELING OF RABBIT LEPTIN

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Leptin protein is a peptide hormone and molecular weight is 16-kDa. Leptin protein was seen as a cytokine formed by adipocytes whose main purpose was to work against obesity. Leptin protein reaches the central and peripheral nervous systems once produced into the circulation, acts by binding with leptin receptor and activating it, which balancing the appetite and food ingestion, discharge of insulin, basal metabolism, bone mass and reproductive role. Recent work was conducted to sequence analysis; multiple sequence alignment and phylogenetic tree analysis. We have also predicted the secondary structure and 3D homology molecular model structure of rabbit leptin protein. Rabbit leptin protein consists of 167 amino acid residues. The multiple sequence analysis of rabbit leptin have found that out of 167 amino acid residues 68 positions are conserved amino acid residues in which 'Leu' was found conserved amino acid residue in leptin protein family. Phylogenetic tree analysis has shown that close relationship between human and rabbit leptin. We have also predicted the secondary structure of *Oryctolagus cuniculus* (Rabbit) leptin protein. These predictions have shown that most of the rabbit leptin consists of alpha helix. *Homo sapiens* (Human) pdb id: 1AX8_A was used as the template, which have shown highest sequence identity 83.33% with rabbit leptin protein. 3D homology molecular model of rabbit leptin protein consists of only alpha helix.

SEQUENCE ANALYSIS, MULTIPLE SEQUENCE ALIGNMENT, PHYLOGENETIC ANALYSIS, AND SECONDARY STRUCTURE PREDICTION, AND 3D- HOMOLOGY MOLECULAR MODELING OF SHEEP RESISTIN

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Resistin is a recently discovered circulating protein, which has no homology to any identified hormone, cytokine, or other intercellular signaling molecule. Resistin molecular weight is 12.5 kDa, rich in cysteine and consists of 108 and 114 amino acids in humans and in mice respectively, with a signal peptide of 17-amino acid, a conserved C terminus and a variable region consists of 37 amino acids. In rodents, it is almost exclusively expressed in white adipocytes. While in human circulating white blood cells such as neutrophils and monocytes produces and macrophages expresses and secretes it. Resistin involve in main function is control of energy homeostasis, inflammation and resistance of insulin. In this study, current work was conducted to analyze the multiple sequence alignment, phylogenetic tree, prediction of secondary structure and homology model of sheep resistin protein. Multiple sequence alignment of sheep resistin protein family have shown conserved residues at position 51cys, 58Gly, 60Lue, 63Lys, 72Cys, 74Cys, 75Gly, 78Cys, 79Gly, 80Ser, 97Asp and 99Thr. Analysis of phylogenetic tree have shown, close relationship of sheep resistin protein with belonging, *Ovisaries*, *Capra hircus*, and *Pantholophodgsonii* indicating that they share a common ancestor. Secondary structure prediction have shown that sheep resistin consists of one major helix, one minor helix, two

major beta sheets and four minor beta sheets. sheep resistin have shown highest sequence identity 59.63 with the target sequence mouse resistin protein pdbi.d; 1rgx.1.A. Homology model of sheep resistin consist of alpha helix and beta sheets.

**QUERCETIN: A SYNERGIST TO ORGANOPHOSPHATE PESTICIDES AGAINST
ADULT AND LARVAE OF *TRIBOLIUM CONFUSUM***

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Stored grain pest is a major issue throughout the world, especially due to the emergence of resistance to pesticides in insects. In Pakistan and other developing countries, grain losses are very high due to pest infestation. Development of a new pesticide incurs hefty investment, however, improvement in the efficacy of existing pesticide may be inexpensive. Purpose of the study was to evaluate the effectiveness of natural phytochemical, quercetin as synergist to organophosphates (OPs) against stored grain pest, *Tribolium confusum*. Three organophosphates, paraoxon-ethyl, tetraethyl pyrophosphate and malathion were used. Feeding or contact method was used. Experiments were repeated for five times. Mortality data were recorded for ten days and analyzed statistically. The results revealed up to 2-folds increase in the toxicity of tested organophosphates when quercetin was used as synergist. Quercetin, however, did not produce significant mortality of beetles when used alone. It is concluded from the study that quercetin may be a potent synergist for organophosphate pesticides. However, dose and concentration regimes may vary with structurally divergent groups of organophosphates. In addition, quercetin being of botanical origin may be considered as safer substitute to the synthetic chemical synergists.

**VALIDATED HPLC METHOD FOR THE DETERMINATION OF BERBERINE AND BERBAMINE
IN RAW HERB SUMBUL (*BERBERIS LYCEUM ROYLE*), ITS EXTRACT AND INSECTICIDAL
ACTIVITIES AGAINST COMMON RESISTANT INSECT *TRIBOLIUM CASTANEUM***

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Medicinal plants have been found promising in treating diseases throughout the world. Herbal treatments are preferred over synthetic drugs due to fewer side effects as reported otherwise in terms of adverse drug reactions, drug-drug interactions and drug resistance so far. Pakistan has plenty of natural resources and is well known for their diverse and valuable medicinal plants. *Berberis lycium* is highly medicinal plant present widely in Pakistan and other countries. In this study Methanol and Ethanol extracts of root, stem and leaves of *Berberis lycium* were extracted through Soxhlet Method. Alkaloids were isolated through High-performance Liquid Chromatography (HPLC) by using sil C18 column with Acetonitrile and Potassium dihydro phosphate as Mobile phase. The elution rate was 1.0ml/min and the detection was monitored at 346nm. High-performance liquid chromatography (HPLC) analysis showed berberine in crude extract of stem but root crude extracts contain high amount of Berbamine and low amount of Berberine, while leaf extracts show negative results. To test the insecticidal activities in this study against the most common and pest resistant insect *Tribolium castaneum* which shown good results with 20% concentration of ethanolic and methanolic crude extracts of *Berberis lycium* with Melathion combination. The insects were tested with 20% methanolic and ethanolic extracts of root stem and leaves alone and combination of extract with different concentrations of Malathion. Combination of extracts of stem and root showed maximum mortality rate as compare to crude extracts alone. Our extracts actually not kill the insects; it can make them sensitive to any insecticide. After Applying our extracts insects can be killed from any insecticide, but leaf extracts again showed no promising results.

GENETICAL EPIDEMIOLOGY OF DIFFERENT TYPES OF HEPATITIS IN POPULATION OF DISTRICT JHANG, PAKISTAN

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Hepatitis genetics and prevalence was evaluated by this planned study carried out on patients of district hospital Jhang. For this purpose, 600 patients participated and they were interviewed by using data sheet for data collection of different parameters and data was analyzed statistically by percentage, mean, standard error, and chi-square test. Hepatitis C was occurring frequently in study population. Male and female patients were equally affected with disease ($P>0.05$). Married patients recorded with higher percentage of hepatitis as compared to unmarried. 35-39 years of age patients were with highest prevalence of hepatitis. So, significant relationship ($P<0.05$) was found between age and hepatitis prevalence. At diagnosis of disease, age male and female patients were 27.66 ± 0.67 and 25.67 ± 0.67 respectively. Patients of 0-4 year's difference of maternal and paternal age were observed with highest occurrence of hepatitis (74.33%). Higher number of patients (36.5%) was recorded with hepatitis B positive blood group. However, hepatitis prevalence does not depend on blood group for prevalence of disease ($P>0.05$). The result revealed that hepatitis prevalence was more in rural population (60.70%) than urban population (39.3%). Highest prevalence of hepatitis was observed in 2nd birth order (17.5%), than 1st (16.2%) and 3rd birth orders (16.2%). Hepatitis prevalence was significantly affected ($P<0.05$) by education, increasing level of education of patient and their parents decreases the hepatitis. Consanguinity had a significant effect ($P>0.05$) for hepatitis prevalence, hepatitis was more observed in first cousin marriages. Ethnic group Sial (5.5%) was with highest prevalence of hepatitis. It was concluded that hepatitis prevalence was more in patients who were married, with B+ blood groups, belong to rural area, and Sial surname, with low income and low qualification level.

SEASONAL VARIATIONS IN SUSCEPTIBILITY OF SPIDERS AGAINST SOME INSECTICIDES

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Use of natural enemies to control insect pests in agro-ecosystem is usually constrained by many natural stressor like temperature, humidity, pathogens, and many toxicants including insecticides. The interactions of these stressor with toxicants can be complex and modify the direct influence of toxicants or their biochemical pathways in the organisms. Spiders are known as natural predators of insect pest in many agro-ecosystems. Use of insecticides not only kill the pests but also effect spider population residing in the field. Present study was designed to investigate the acute toxicity of Emamectin Benzoate and Lambda Cyhalothrin on *Oxyopes javanus* population in different seasons. Laboratory bioassays were conducted by applying various concentrations of insecticides on *O. javanus* using Dip method in three seasons, spring, summer and Autumn. The LC_{50} values were calculated using mortality data for each insecticide after 24 hours. Results showed significant positive relationship between molarity and concentration of insecticides in every season. For each insecticide, highest mortality was recorded in summer while lowest in winter. Male *O. javanus* were more susceptibility to both insecticides than female in every season. Lambda Cyhalothrin was more toxic to *O. javanus* as compared to Emamectin Benzoate in every season. Our results highlighted the effect of temperature on the susceptibility of insecticides. So the field application rate of insecticides should varies depending upon the environmental conditions of the area.

**TOXICOLOGICAL AND ULTRASTRUCTURAL EFFECTS OF TEBUFENOZIDE, ON
ANTICARSIA GEMMATALIS (HÜBNER) (LEPIDOPTERA: NOCTUIDAE)**

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The velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), is one of the most important pest of soybean crop, ranging from the Gulf Coast area of the United States to Argentina. Tebufenozide, a novel nonsteroidal ecdysone agonist among many other insecticides, was used to control this pest. The LC₅₀ values (3.86 mg/L) calculated by probit analysis, showed significant damage to midgut cells of this insect. However, morphological changes in midgut cells were observed under light and transmission electron microscopes after treating the larvae with LC₅₀ of tebufenozide. Histological sections were monitored for 24, 48 and 96 hours while, transmission electron micrographs were examined after 24 hours of treatment. Results revealed that damage to cells was significantly increased with the passage of time. Besides, the dissection of treated caterpillars showed a clear damage to striated border with release of protrusions to the midgut lumen, damaged nuclear membrane and nucleus with crumpled chromatin. In addition, the autophagic vacuoles with accumulation of granules in cytoplasmic region were prominently observed. Moreover, tebufenozide caused enough molecular damage to mitochondria by reshaping it in to nanotunnels mitochondria, which could be an obvious evidence that tebufenozide induces substantial molecular damages to cells, thus resulting in cell death.

**OCCURRENCES OF APHIDOPHAGOUS HOVERFLIES IN COASTAL
AREAS OF SINDH, PAKISTAN**

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A variety of Syrphidae species occur in agroecosystems. The occurrences and species composition of aphidophagous hoverflies was assessed in various localities of coastal areas of Thatta and Badin, Sindh, Pakistan during January -April 2017. In these areas different crops are cultivated like vegetables; brassica, tomatoes, cabbage, wheat, green chili, okra and sunflower. The floral resources enhance the longevity and fecundity of adult dipterous flies. Aphidophagous hoverflies are one of the most significant groups of hoverflies and naturally predators play vital role in pest management, because their larvae enjoying feeding over insect pest, (aphids, Jassids, Thrips) while adult are good pollinators. The adults of two species, of genus *Epeodes* *E. corollae*, *E. luniger* were collected from different agriculture fields of Badin and Thatta by Insect hand net and Malaise trap and brought to an Insectary, Department of Zoology, University of Sindh, Jamshoro, for identification and preservation. As a result 231 individuals of two species of genus *Eupeodes*, *E. corollae*, *E. luniger* were identified. Among these two species, *Eupeodes corollae* was most abundant with 187 specimens. Monthly population revealed that the maximum abundance of these flies was observed in March (92) followed by April (69) while minimum in January (26). These results suggest that there was positive effect of temperature and number of host plants and aphid colonies on the prevalence of these aphidophagous hoverflies. Current Study is a part of Project No: 20_3838/NRPU/R&D/HEC/14 Higher Education Commission Islamabad Funded.

DIVERSITY OF APHIDOPHAGOUS HOVERFLIES (DIPTERA: SYRPHIDAE) IN AGRICULTURAL FIELDS OF MANSEHRA, KHYBER PAKHTUNKHWA, PAKISTAN

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The present study revealed the biodiversity (richness, abundance, evenness and diversity) of aphidophagous hoverflies belonging to sub-family Syrphinae from agricultural fields of Mansehra, Khyber Pakhtunkhwa, Pakistan during January-November 2017. A total of 539 specimens of hoverflies belonging to five species consists of 4 genera were recorded from different localities of Mansehra. Diversity indices of Shannon- Wiener's diversity index and Simpson's reciprocal index were used to calculate hoverflies species diversity in different agriculture field of Mansehra. The calculated values of diversity indices showed that highest diversity was recorded from Baffa followed by Shinkari and lowest in Dhodial. The spirit of present study was to monitor the month wise prevalence of aphid eating hoverflies, the results shown that the maximum abundance of these flies were recorded in March and April, whereas; minimum in September. The collected species were *Episurphus balteatus*, *Eupeodeus corolla*, *Ischiodon scutellaris* and *Sphaerophoria scripta*. Among these species, *Episurphus balteatus* was found most prevalent while *Eupeodeus corollae* was found least prevalent species in studied areas.

PREVALENCE OF APHID SPECIES ON WINTER CROPS (HOMOPTERA: APHIDIEA) IN TANDOJAM AND SHAIKH BHIRKIO

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Green peach aphid is cosmopolitan and found throughout the world. It is injurious to the plants mainly due to its virus transmitting ability to the plants. Green peach aphid attains a very huge range of host plants, it feeds over 40 plant families. Hyderabad district is famous for its horticulture and agricultural crops. Present study was carried out from September-December 2017 in Hyderabad district in which different fields were visited including brassica, cabbage, okra, spinach, chilli, grasses and tomato. Aphids were collected either by removing them with an ordinary camel-hair brush or by jarring the plants on white paper sheets from a wide range of host plant. A few aphid colonies along with the attacked plant parts were also brought to the laboratory. As a result, 3 species belonging to 3 different genera of 1 family Aphididae were identified i.e. *Myzus persicae*, *Brevicoryne brassicae* and *Aphis fabae*. Out of these 3 *Myzus persicae* was most prevalent species because it is polyphagous in nature and also possess a huge host plant range throughout the year while *Aphis fabae* was observed least prevalent species of aphid. It was observed that all the stages of Aphid species preferred juicy and soft parts of the plants for infestation. As a total result 7 colonies belonging to 1 species, 1 genus of family Aphididae identified. Green peach aphid was collected from different host plants i.e. Brassica, cabbage, cauliflower, okra, spinach and tomato. The *Myzus persicae* were collected in abundance during the study period from brassica, cabbage and grasses. Maximum number of *Myzus persicae* was recorded from Brassica followed by cabbage and least number was observed from grasses. It was observed that the all stages of *Myzus persicae* preferred juicy and soft parts of the plants for infestation.

Host plant range

Host Plants	Localities	Number of specimens
Brassica	Tandojam	201
Wheat	Tandojam / Husri	158
Cabbage	Tandojam / Mori manger	113
Spinach	Tandojam	97
Cauliflower	Tandojam	42
Tomato	Husri	13
Total		624

OCCURRENCE OF TWO PREPOTENT SPECIES OF GENUS *HYALOMMA*, *SCUPENSE* AND *EXCAVATUM* (PARASITIFORMES: IXODIDAE) IN BUFFALOES OF LARKANA, SINDH, PAKISTAN

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A study was conducted to evaluate the prevalence of two species of genus *Hyalomma* on buffaloes of district Larkana. Different dairy farms of Larkana were visited fortnightly from March to September 2017. 1750 buffaloes were randomly observed among them 877 buffaloes (50.11%) were found infested with ticks. Total 955 specimens were collected and preserved in glycerin filled bottles. The bottles were labeled mentioning the sex of buffalo (male and female) and various parts of body such as head, neck, tail, udder, teats and legs. Specimens were brought to the Entomology laboratory department of Zoology, University of Sindh, Jamshoro, Sindh, Pakistan. Two species *Hyalomma scupense* Schulze, 1919 and *Hyalomma excavatum* Koch, 1844 were identified under a dissecting binocular microscope by using the standard identification keys. Infestation of *Hyalomma scupense* was relatively higher with 56% and it is new species record from Larkana, Sindh as well as from Pakistan than *Hyalomma excavatum* 43.97%.

GROWTH RESPONSE AND FLESH QUALITY OF CHINESE CARPS UNDER THE INFLUENCE OF DI- AMMONIUM PHOSPHATE AND AMMONIUM NITRATE

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Two ponds were stocked with Common carp (*Cyprinus carpio*), Grass carp (*Ctenopharyngodon idella*) and Silver carp (*Hypophthalmichthys molitrix*) at the proportion of 1:2:1 to mounted growth and flesh quality of carps. Pond 1 was treated with Di-Ammonium Phosphate (DAP) and pond 2 with Ammonium Nitrate @ 0.3g N/100 gm of fish body weight per day. Total net fish production shown by pond 1 and pond 2 was 608.975 and 618.335 kg/ha/year respectively. The fish production is more in pond with ammonium nitrate than di-ammonium phosphate by 1.01 times. The water quality parameters were measured on every week. At the end of the experiment the flesh quality of three said Chinese carps was observed. Maximum protein and fat content observed in *C. idella* (17.74% and 5.37% respectively). *H. molitrix* showed maximum carbohydrate and ash contents (2.43% and 26.12% respectively) while the moisture content was maximum in *C. carpio* of 71.29%. Proximate composition showed statistically non-significant ($P>0.05$) results.

A PRELIMINARY SURVEY OF INSECTIVOROUS BIRDS OF LAHORE AND SUBURBS

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A preliminary survey of insectivorous birds of Lahore and suburbs was carried out, from Jan to December 2017. The aim of this study was to record the bird species that chiefly feed on insects. Only those birds were recorded that either only eat insects or insects are a major part of their diet. Birds were observed and photographed with Canon 1300 D camera and 50-250 mm zoom lens. The insectivorous bird species observed during this times period are, bank myna, blue-tailed bee-eater, black drongo, barn swallow, cattle egret, common hoopoe, common babbler, citrine wagtail, Eurasian cuckoo, house sparrow, house swift, jungle babbler, little green bee-eater, paddyfield pipit, pied cuckoo, red-throated flycatcher, striated babbler, spotted owl, tawny pipit, white-browed fantail flycatcher, wire-tailed swallow and yellow-eyed babbler.

CHALLENGES FOR CAPTIVE WILD MAMMALS AT SAFARI ZOO LAHORE BY POST-MORTEM ANALYSIS

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Captive breeding is an important conservation tool. Estimation of births and mortalities are important to test ecological and evolutionary theory. The current study was conducted to estimate birth and mortality rates along with overall challenges that lead to death of mammals at Safari zoo Lahore. Data was collected through stock position and post-mortem reports. Year wise data indicated that there was 25%, 20%, 20%, 17% and 18% birth rate in 2012, 2013, 2014, 2015 and 2016 respectively. While mortalities in respective years were 11%, 8%, 11%, 23% and 47%. Highest mortalities (47%) were recorded in 2016 due to viral infection (Malignant catarrhal fever) in Black buck. Data was analyzed and survivorship curves based on life tables were constructed. Through this analysis, age specific mortality rates along with variations among species were assessed. There were no age specific mortalities in species although significant variations existed among juvenile and adults. Gender based analysis through Mann- Witney U-test revealed that in African lions there were higher mortalities in males than females while in ungulates no effect of gender was observed. Post-mortem reports revealed that most of the deaths were due to respiratory, bacterial and viral diseases. Some other factors like stress, shock and trauma also contributed for the same. The data will provide useful information about status of disease triggering mortality and their rates based on age and gender. It would be helpful in the development of veterinary protocols for healthy breeding of wild animals in captivity.

SCAVENGING ON POISONOUS DEAD INSECTS

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Scavenging is a specialized feeding strategy of various animals using nutrient rich dead bodies of other animals. Many traditional predators were found to feed often on carcasses of animals of diverse size. Living animals possess diverse defence mechanisms against predation, such as chemical, mechanical and behavioural. Among them, ladybirds (Coleoptera: Coccinellidae) are known for their content of alkaloids. Rate of disappearance of fresh carcasses of various small edible and poisonous invertebrates exposed on soil surface in semi-natural habitat will show how much are ladybirds protected chemically and mechanically without the contribution of their behaviour. We placed five types of dead (frozen) prey, each as five individuals, in a square grid (randomized in Latin square) on the soil surface in a semi natural habitat (grassy field margin) in Czech Republic in June 2017. The prey species were: Harlequin ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae), firebug *Pyrrhocoris apterus* (Heteroptera), little mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), woodlouse *Porcellionides pruinosus* (Isopoda: Porcellionidae) and immature cricket *Acheta domestica* (Orthoptera: Gryllidae). We observed regularly which individual prey disappeared during day (experiment starting at 8:00) and during night (experiment starting at 21:00) using headlamp. The identity of scavengers was not recorded, however, we observed some ants (Hymenoptera: Formicidae) and ground beetles (Coleoptera: Carabidae) pulling some of the prey. During day, the median time required for the disappearance of *A. domestica* was 2 hours, of *P. pruinosus* 2.5 hours, of *A. diaperinus* 6 hours, while more than 50 % of *P. apterus* and *H. axyridis* remained untouched within 24 hours. During night, the median time required for the disappearance of *A. domestica* was 1 hour, of *P. pruinosus* 1.5 hour, of *A. diaperinus* 5 hours, while more than 50 % of *P. apterus* and *H. axyridis* remained untouched within 24 hours. Small diurnal invertebrate predators and scavengers as well as birds distinguished strongly between palatable prey (soft and non-toxic cricket and woodlouse), less palatable prey (hard and foul-smelling tenebrionid), and toxic prey (firebug, ladybird). Nocturnal invertebrate predators and scavengers distinguished between the prey properties similarly, and were more active than the diurnal ones.

OPTIMIZING THE PRODUCTION OF XYLANASE BY *ASPERGILLUS FLAVUS* ZGCL17 USING WASTE FIBER SLUDGE**Bushra Kalim*, Nazish Mazhar Ali and Nasir Bashir***Microbiology Laboratories, Department of Zoology, GC University, Lahore, Pakistan*

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Waste fiber sludge (WFS) and wheat bran (WB) were used as a substrate for xylanase production while using *Aspergillus flavus* ZGCL17 isolated from fertile soil. The substrates were washed, dried, milled and wetted with Vogel's medium. The cultures were grown at 31 °C for 5 days. Cell free culture filtrate (CFCF) was subjected to enzyme activity estimation. The fermentation conditions were optimized for xylanase production observing the effects of time course, temperature, pH, substrate concentration, carbon and nitrogen supplements. The optimum culture conditions for the xylanase production by *Aspergillus flavus* ZGCL17 were observed to be on the 7th day of incubation at 30 °C and pH 5 with 4% substrate concentration. *Aspergillus flavus* ZGCL17 produced a high level of xylanase under submerged fermentation, which may have potential industrial application.

PROSPECTIVE ANALYSIS OF INFLAMMATORY MARKERS IN THE HYPERTHYROID SUBJECTS OF CENTRAL PUNJAB**M. A. Iqbal¹, Z. Naseem¹, N. Roohi¹, and S. Ahmad²**¹*Physiology/Endocrinology Laboratory, Department of Zoology, University of the Punjab,**Quaid-e-Azam Campus, Lahore-54590, Pakistan.*²*King Edward Medical College University, Lahore*

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Thyroid is a prominent endocrine player in maintaining homeostasis of the body by the synthesis of vital endocrine hormones, tetra-iodothyronine (T4) and tri-iodothyronine (T3). Any alteration in the actions of T3 and T4 (hypo or hyper activity) influences the normal metabolic pathways of both embryonic and adult life. To investigate the responsiveness of the inflammatory markers like C-reactive protein (CRP) and Interleukin-6 (IL-6) and Homocysteine (Hcy) in hyperthyroid subjects after antithyroid treatment, attaining euthyroid state. A total of 179 subjects comprising both genders and full filing the exclusion and inclusion criteria were recruited for this prospective investigation. Sixty healthy controls (40 females and 20 males), thirty four subclinical (21 females and 13 males), fifty overt hyperthyroid (35 females and 15 males) and thirty five follow-up (25 females and 10 males) subjects having 3 months of anti-thyroid therapy (Carbimazole) were enrolled. Analysis of the thyroid profile and inflammatory markers was performed by RIA and ELISA, respectively. One-way ANOVA was applied for the statistical analysis, while significance ($P < 0.05$) of means was compared by "Student Newman Keuls" (SNK) test. Pronounced elevation ($P \leq 0.01$) of CRP in overt hyperthyroid state was noted in comparison with control. While, non-significant difference was observed in all other intergroup contrasts. Both subclinical and overt hyperthyroid groups manifested prominent elevation ($P \leq 0.01$ and $P \leq 0.001$, respectively) of IL-6 when compared with controls. Whereas, follow-up group depicted prominent ($P \leq 0.01$) elevation of IL-6 as compared with controls, even after having anti-thyroid treatment. In case of Hcy, both subclinical and overt group manifested pronounced ($P \leq 0.05$ and $P \leq 0.001$, respectively) reduction as compared to controls. Moreover, follow-up patients presented a significant ($P \leq 0.05$) improvement of Hcy level after anti-thyroid treatment. The study concludes that hyperthyroid states markedly influences the biosynthesis of on inflammatory markers. CRP and IL-6 has a positive correlation with FT₄ and FT₃ levels. Whereas, Hcy demonstrated a negative correlation with thyroid hormones. This study was funded by Higher Education Commission, Pakistan.

MOLECULAR PHYLOGENETIC STUDIES OF SOME PASSERINES OF PAKISTAN BASED ON CYTOCHROME OXIDASE SUBUNIT I

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A comprehensive inventory of all forms of life is basic need of scientific study of evolution and biodiversity. The international "Barcode of life project" is an attempt to identify Earth's biodiversity at species level, using short signature DNA sequences of different taxa. DNA barcodes of birds are currently available for 41% of known species worldwide and is rich data source to answer evolutionary questions and offer high quality data for species identification but no research in this area has been done in Pakistan. The current study was planned to employ DNA barcoding to conduct molecular identification through amplification of Cytochrome Oxidase Subunit I (COI) and to generate phylogenetic tree to reveal the evolutionary and genetic relationship among different species of Passeriformes of Pakistan. Samples were collected from different parts of Pakistan and their preserved skins were processed for DNA extraction. Folmer region of COI gene comprising ~650 bp was amplified and sequenced and data was analyzed by BioEdit software 3.2 versions and was further analyzed using BLAST on NCBI for identification of species. CLUSTAL W and MEGA7 software were used for inference of phylogenetic relationships between different species by the neighbor joining (NJ) using Maximum likelihood method, of 47 amino acid sequences and estimation of nucleotide distances between sequence pairs and diversities using Kimura-two-parameter. Close sequence matches (<2% divergence) were detected and barcoding was proved to be effective in identifying Passeriformes of Pakistan.

CLICKER TRAINING OF SEMI CAPTIVE ASIATIC BLACK BEARS: A FOCAL SCAN STUDY

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In Pakistan, Asiatic black bears (*Ursus thibetanus*) are poached, used for bear baiting and dancing, thus, forced to live a miserable life. Balkasar Sanctuary situated at Chakwal is the only sanctuary in Pakistan working for bear rescuing and rehabilitation. However, when bears are rescued and separated from their previous owners, they show stereotypic behavior which also hinders their interaction with sanctuary managers. Therefore, current study was conducted in Balkasar Sanctuary to use clicker training as a tool to reduce stereotypy and study the behavioral changes of Asiatic black bears (*Ursus thibetanus*). Ethograms were made for active, passive and abnormal behaviors for focal sampling of bears. Results showed positive behavior with increase in active behavior of bears and there interactions with managers of sanctuary. Thus, concluding that these training may bring long term changes in behavior of semi captive bears.

COST EFFECTIVE PROTOCOL FOR GENOMIC ISOLATION FROM SCAT SAMPLES

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According to IUCN, there are number of wildlife species which are threatened due to habitat loss and degradation, tree line shifting and various anthropological activities with dire need to be conserved. For this very purpose, molecular analysis plays vital role, for which animal samples are required i.e. invasive or non-invasive. Invasive sampling, however, is quite challenging due to threats faced by species, thus, fecal remains

(non-invasive samples) of animals are source of gDNA required for molecular studies. Unfortunately, most of laboratories have to use expensive kits for this DNA isolation as very low quality DNA is obtained by manual extraction. Therefore, in order to improve the quality and quantity of gDNA extraction from fecal samples and for successful PCR amplification a protocol is designed in which upper layer of feces was removed, washed with PBS and then after processing through Phenol and Chloroform passed through spin column filters along with binding buffer. Through this protocol 67% gDNA (30 samples out of 50) was extracted, later on amplification was done by using Mini-barcode which is an excellent marker for identification of mammals which involved 650 bp COI gene fragments which can amplified smallest base pair of 90 bps from degraded fecal DNA. Sequencing analysis and blast results revealed that 60 % samples were of expected species 40 % were of other species including fungi 26.6 %, hare 8.9 % and Himalayan Tahr 4.4 %.

OPTIMIZATION OF INDIVIDUAL VENTILATION CAGES (IVC) AND TUMOR INDUCTION IN *IN VIVO* SPECIMENS

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Oncological studies emphasizes on environmental refinement which helps to enhance the physical and psychological behavior of animal along with the assurance of sterilized environment. Animal keeping, breeding and experimentation no doubt is a hard task to compensate but the individual ventilation cages (IVC), an attractive housing regime made it much easier especially for laboratory rodents. Effective reduction of ammonia, safe handling and safety from high degree of contaminants with relative availability of favorable environmental condition for experimental animals are the basic benefits of IVC. Keeping in view the effectiveness Bioresource research centre, Islamabad organized an IVC chamber with successful optimization of conditions i.e. temperature (25±3), pressure (1±0), Humidity (38±5%) and airflow. Moving steps forward the successful induction with Carbon tetrachloride and cell lines were done within a limited duration of 6 months. Designing of the study was based on three groups having 14 specimens separated on the basis of sexes out of which 8 were administrated with CCL₄ dosage Intraperitoneal with volume of 1mL/kg twice a week by using 24 guage syringe while 4 rats were administered 2 doses of MCF-7 cell lines fortnightly at the tissue pad of breast. The confirmation was done by Hematology, Liver Functioning and Kidney functioning tests and AlphaFeto protein. Production of tumor mass caused abnormal growth cells, results in rupturing and degradation of cells. This will be helpful in future for analysis of drug potency check against oncological cells and their cytotoxic effects on animal organs.

PHYLOGENETIC ANALYSIS OF FAMILY COLUMBIDAE (AVES) OF PAKISTAN BASED ON CLASSICAL AND MOLECULAR TAXONOMY

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Columbidae (class Aves) is family of doves and pigeons, with 310 species reported worldwide. Many species belonging to this family are listed in IUCN red list. Under classical taxonomy morphological traits have been has been used for species identification. Nowadays use of molecular biology techniques, such as DNA barcoding, is rising and is considered more reliable for species identification, yet it needs the support of traditional classical taxonomy. The current study was designed to compare molecular and classical taxonomy techniques and to develop phylogenetic tree, using members of the family Columbidae in Pakistan as a model. For this purpose, field collected samples were identified on morphological basis (using traditional keys) as well as by using COI region of mtDNA. For molecular analysis DNA was extracted from tissue and COI gene was

amplified using appropriate primers. PCR products were further sequenced and Phylogenetic trees were constructed which showed reliable linkages between different species of family columbidae.

OPTIMIZATION OF TANNASE PRODUCTION BY *BACILLUS AMYLOLIQUEFACIENS* IN SUBMERGED FERMENTATION

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Ten bacterial isolates isolated from fish gut were screened for tannase production. Among these, four species, *Roultella ornithinolytica*, *Klebsiella oxytoca*, *Enterobacter aerogenes* and *Bacillus amyloliquefaciens* expressed greenish zones around their colonies. *B. amyloliquefaciens* showed highest tannase production (1.27 IU/ml) under unoptimized conditions and was selected for further work. During one factor at a time optimization of physical parameters, 37 °C incubation temperature, 5 pH, 1% inoculum size and 24 hours incubation period yielded maximum tannase. To screen the significant medium components, 12 experimental runs of Plackett-Burman design for six variables (tannic acid, K₂HPO₄, CaCl₂, MgSO₄, NH₄NO₃ and yeast extract) were carried out. From these experimental runs, the enzyme assay results were analyzed using multiple regression. Three variables *i.e.* tannic acid, CaCl₂ and yeast extract showed significant impact on tannase production. Concentrations of these variables were optimized using Box–Behnken design (BBD). Results of 15 experimental runs of BBD showed maximum tannase production corresponding to 0.5% tannic acid, 0.1% CaCl₂ and 0.275% yeast extract. The highest tannase stability was measured at pH 7, 0.5% substrate concentration and 40 °C. Further work is required to screen the potential of this bacterium for reducing tannic acid contents of complex natural substances which can be used as ingredients of fish feed otherwise.

EXPOSURE ASSESSMENT OF AMBIENT PARTICULATE MATTER ON ROADS OF LAHORE, PAKISTAN

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Exposure to particulate matter along roadsides can result in both short-term and long term adverse consequences upon health of the travelers. The current study determined the exposure level of commuters by recording the ambient levels of TSPM, PM_{2.5} and PM₁₀ along three major roads of Lahore city (Ferozpur Road, Canal road, and Lahore Ring Road). Sampling was conducted from 1st -3rd December, 2016 starting from 9:00 am to 12:00 pm along each route on an open vehicle. Seventy samples were collected with the help of a high Volume Dust Sampler and Gradko DC1700 air particle counter. Meteorological parameters were also recorded after every 2 km intervals along the entire route. Mean levels of TSPM, PM_{2.5} and PM₁₀ were documented to be 36 ± 8.7, 1446 ± 336.28, and 16.9 ± 4.71 respectively. Significant correlation was observed between particulate matter and relative humidity. Likewise same was the case with PM_{2.5} and lead. On the contrary, non-significant correlation was observed between temperature, particulate matter and heavy metal concentrations. Principal Component Analysis was used to identify the sources of heavy metals recognizing three source contributing factors including vehicular emissions, industrial emissions, and natural sources. Source of Zn and Pb was identified as traffic related emission; Cd and Cu were identified as industrial pollutants whereas Cr and Ni were generated by natural activities. Results suggested that anthropogenic activities were major source of heavy metals, TSP, and particulate matter.

CHEMICAL AND UV MUTAGENESIS FOR ENHANCED PRODUCTION OF CHITINASE FROM *BACILLUS SUBTILIS*

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This work was undertaken to enhance the production of industrially important chitinases by indigenous chitinase producing bacterium, *Bacillus subtilis* using rice straw as agro-industrial waste. To improve the chitinase producing efficiency of selected wild type strain, random mutagenesis were carried out employing physical (ultra-violet radiation) and chemical (ethyl methane sulfonate and ethidium bromide) treatments. Mutant strains were screened on the basis of chitinase production. During physical treatment, exposure time and distance of expressed cells from source were optimized. In case of chemicals treatment, chemicals concentration and exposure time were optimized. Single-stage, di-stage and tri-stage mutagenic treatments were carried out to enhance the enzyme production. Mutant strain were found more efficient for yielding chitinase as compared to wild type strain. Chemical treatment showed better results as compared to physical treatment. The mutants appear promising for provision of economical and large scale production of chitinases.

HABITAT SUITABILITY AND MOVEMENT CORRIDORS OF GREY WOLF (*CANIS LUPUS*) IN NORTHERN PAKISTAN

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Habitat suitability models are useful to understand species distribution and to guide management and conservation strategies. The grey wolf (*Canis lupus*) has been extirpated from most of its historic range in Pakistan primarily due to its impact on livestock and livelihoods. We used non-invasive survey data from camera traps and genetic sampling to develop a habitat suitability model for *C. lupus* in northern Pakistan and to explore the extent of connectivity among populations. We detected suitable habitat of grey wolf using a maximum entropy approach (Maxent ver. 3.4.0) and identified suitable movement corridors using the Circuitscape 4.0 tool. Our model showed high levels of predictive performances, as seen from the values of area under curve (0.971 ± 0.002) and true skill statistics (0.886 ± 0.021). The main predictors for habitat suitability were distances to road, mean temperature of the wettest quarter and distance to river. The model predicted ca. 23,129 km² of suitable areas for wolf in Pakistan, with much of suitable habitat in remote and inaccessible areas that appeared to be well connected through vulnerable movement corridors. These movement corridors suggest that potentially the wolf range can expand in Pakistan's Northern Areas. However, managing protected areas with stringent restrictions is challenging in northern Pakistan, in part due to heavy dependence of people on natural resources. The habitat suitability map provided by this study can inform future management strategies by helping authorities to identify key conservation areas.

AIR QUALITY IN HOSPITALS OF DEVELOPING COUNTRY: A CASE STUDY IN PAKISTAN**Nimra Afzal¹, Zulfiqar Ali¹, Zaheer Ahmad Nasir², Sean Tyrrel², Sidra Safdar³ and Komel Ahmad¹**¹*Environmental Health and Wildlife, Department of Zoology, University of the Punjab, Lahore*²*School of Energy, Environment and Agrifood, Cranfield University, Cranfield, Bedfordshire MK43 0AL, UK*³*University of Veterinary and Animal Sciences, Lahore, Pakistan*

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This study investigates air quality in Orthopaedic operation theatres, wards and emergencies of six Government, Private and Trust hospitals of Lahore, Pakistan. Measurements were made for the general air quality indicators, such as PM, VOC's, CO₂, relative humidity and temperature. The aim of the study was to provide an indication of impact of particulate pollution on hospital environment and relate particle characteristics to the operating parameters of hospitals. Mean levels of PM_{2.5}, PM₄, PM₁₀ and PM_{Total} in hospitals indoors were 94+55, 99+56, 117+63 and 143 +75µg/m³ respectively, while the mean ambient levels of PM_{2.5} were 547+ 67 µg/m³. The average PM_{2.5} I/O ratio of 0.18 suggest increase possibility of infiltration from outdoor sources. Regression analysis showed varied relation between hospital indoor and ambient air (R²: 4 to 80%). Current investigation conclude that indoor levels of PM_{2.5} are related to ambient levels, indicating outdoor particulates as an important contributor to indoor particulates, depending upon the proximity of the site to road and performance of ventilation/filtration systems. Increase levels of particulate concentration can result in increased number of carriers for organic and microbial aerosols and eventually in increase risk of airborne infection. Therefore, controlling particulate matter concentration in the hospitals have direct implication for microbial and infection control measures.

**MONITORING THE CAPTIVE BEHAVIOR AND ACTIVITY PATTERN OF CARNIVORES:
PANTHERA LEO AND PANTHERA TIGRIS IN LAHORE ZOO****Noor-ul-Ain and Zulfiqar Ali****Department of Zoology, University of the Punjab, Lahore, Pakistan*

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Tigers and lions in the wild are nocturnal nonhuman animals that may hunt and mate opportunistically during daylight hours. In captivity, they spend most time on exhibit sleeping or pacing. This study sought to better understand their activity budget. The daily behavior patterns of 4 Bengal tigers, 8 African lions and 4 lion cubs in different housings were observed. The study was conducted in Lahore Zoo which included 6 hours per day observations of animal 's daily activity budget from 8:00-10:00am, 12:00-2:00 pm and 4:00-6:00 pm in the months of February and June for 10 days in each month. During the study, moving, resting, feeding, auditory, aggressive and excretory activity patterns were recorded by scan sampling method. The proportion of scans the large felids spent engaged in different activities varied by time of day and environment. Lions and tigers spent most of their time in resting. There was significant variation in daily activity patterns of lions and tigers in both seasons. This study will be helpful in future researches on captive activity patterns of carnivores.

CLINICOPATHOLOGICAL PATTERNS OF UTERINE LEIOMYOMA PATIENTS**Sundas Akram, Nabila Roohi and A. Akmal***Department of Zoology, University of the Punjab, Quaid-e-Azam campus, Lahore-54590*

Uterine leiomyomas are highly frequent gynecological neoplasms with major public health impact. The objective of study is to systematically analyze clinico-pathological patterns, electrolyte profile and marker enzymes in leiomyoma patients. The study comprised of 44 control subjects and 46 leiomyoma patients, aged 21- 50 years. Clinicopathological findings, anthropometric parameters and detailed history of disease were recorded through a standardized questionnaire followed by uterine ultrasound examination. Blood samples were drawn for the measurement of serum electrolytes and marker enzymes levels by using commercially available

kits. Among clinico-pathological patterns, high frequency of leiomyoma (43.5%) was found in the age between 30-40 years. Intramural uterine leiomyomas were more common type of leiomyoma (61%) and majority of leiomyoma were found single (52%). Menorrhagia was common clinical manifestation with 63% leiomyoma cases. Family history of fibroid was observed in 26% leiomyoma women. A significant increase in body mass index (BMI), diastolic blood pressure (DBP) and significant decrease in parity was recorded in leiomyoma subjects in comparison with controls. Serum electrolytes analysis revealed a significant increase in the concentrations of chloride (Cl^{-1}) as well as calcium (Ca^{+2}) and significant decrease in potassium (K^{+1}) concentration in leiomyoma group when compared to the controls. While, non-significant difference was recorded in serum sodium (Na^{+1}) level between comparable groups. Results of marker enzymes analysis manifested significant elevations in the serum levels of acid phosphatase (ACP), aspartate transaminase (AST) and alanine transaminase (ALT) in leiomyoma patients in relation to controls. While, non-significant variations were found in serum alkaline phosphatase (ALP) levels between comparable groups. Elevated serum Na^{+1} , Cl^{-1} and Ca^{+2} and reduced K^{+1} concentrations in leiomyoma patients characterize increased estrogen level, responsible for leiomyoma growth and serum levels of ALT, ACP and AST are viable diagnostic markers of uterine leiomyoma.

SEASONAL MONITORING OF INDOOR/OUTDOOR PARTICULATE MATTER WITH REFERENCE TO LUNG CAPACITY OF FARMERS OF UPPER HUNZA, GILGIT BALTISTAN PAKISTAN

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For the present study ambient concentrations of particulate matter fractions (PM_{10} , $\text{PM}_{2.5}$, PM_{10} , PM_{total}), total suspended particles (TSP), indoor VOCs, seasonal variations, indoors, outdoor, seasonal heavy metal variations and indoor vs. outdoor bacterial CFU, were measured from for one year in upper Hunza, GB, Pakistan. The data consisted of 48 hours real time monitoring of all PM fractions indoors and $\text{PM}_{2.5}$ monitoring outdoors. Chemical analysis for heavy metals detection and bio aerosol analysis was carried out after volumetric filter sampling of indoor and outdoor air in different seasons. It was found that winters had the highest concentration for all PM fractions while summer concentrations were lowest. By applying univariate analysis the results indicated that there was significant difference between 24 hr average PM values of the seasons. ($F=636.366$, $p<0.05$). All of these average 24hrs values either indoors or outdoors for all the seasons were exceeding the standards set by USEPA and AAQCs. The TSP showed a seasonal difference with highest TSP value during winters and low concentration during summers. VOCs were highest in summer (2155.25 ± 914) and lowest in winters (938.83 ± 369.11). After bioaerosol analysis six types of bacteria were being identified. The metal analysis results showed that the concentrations of all metals (Zn, Cd, Cr, Cu, Ni and Pb) were exceeding the AAQCs and USEPA standards. The spirometric test results showed that the predicted values were higher than actual values. The results suggest that more protective measures needs to be taken during farming and better ventilation system is required in kitchen/living rooms during winters.

GLP-1: A POWERFUL PHYSIOLOGICAL INCRETIN. SYSTEMATIC REVIEW OF THE LITERATURE

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Food intake, especially carbohydrate, release incretin which is an endocrine transmitter, produces in gastrointestinal tract and stimulate insulin secretion. Among various endocrine modulators, GLP-1 is more effective in stimulating the release of insulin and more powerful regulator of physiological function. GLP-1 executes their action through structurally diverse G-protein coupled receptors (GPCRs). Mainly GLP-1 receptors expressed in lungs, α and β cells of pancreatic islets and nervous system. Peripheral tissues,

gastrointestinal tract, and extra pancreatic tissues i.e., vascular smooth muscle, kidney and heart also contain high affinity receptors for GLP-1. The aim of this systematic review was to gather the available published evidence of the functions performed by GLP-1 through the activation of its receptor GLP-1R on various organs. Present study summarizes the effect of GLP-1 on beta cell apoptosis, on weight loss in diabetic patients, in regulations of pancreatic secretions, GLP-1 as neurotransmitter, in stress response of hypothalamus and in regulation of cardiac functioning. A comprehensive review of all relevant studies published in English language was performed using established methods for systematic review research. The inclusion and exclusion criterion defined in perspective protocol was used to analyze the literature. Studies including at least one function of GLP-1 were included. Tables including key information from each accepted study were assembled and a qualitative synthesis of all included studies was performed. Statistical analyses were not planned and possible. Our findings showed the increasing evidence in published literature pointing towards functions of GLP-1. Studies of this review concluded that GLP-1 receptor signaling helps prevent beta cell apoptosis and conserve function and morphology of human islet as well. The significant effect of GLP-1R signaling in weight loss in diabetic patients was proved by previous studies. The long term use of GLP-1R agonists reduces cardiovascular and renal complications in diabetic patients as well. Significant evidence was found in previous literature for the effect of GLP-1R on pancreatic secretions. The secretions of many hormones like trypsin, lipase and glucagon inhibited significantly while the increase in level of insulin and somatostatin was reported in many studies. GLP-1R has prominent role in cardiac functioning and increase heart rate considerably. Based on the vast impacts of GLP-1 on physiological functions many GLP-1RAs can be made to. That can increase the healthy life span.

MORPHOLOGICAL VARIATION AND ECOLOGICAL CORRELATES OF ANURAN TADPOLES IN RAWALPINDI/ ISLAMABAD AREA

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We carried out present study to examine variation in morphological characters of anuran tadpoles and to correlate occurrence and abundance of tadpoles of various anuran species with their habitat features (elevation; vegetation; type of substrate as rocky or muddy; surrounding cover; wetland type as lentic or lotic; water quality: pH, water temperature, total dissolved salts, electric conductivity, salinity and environmental variables: air temperature, humidity, wind speed, wind chill and heat index) in the areas of District Rawalpindi and Islamabad Capital Territory from July 2016 to August 2017 using standard procedures. We have presented our findings on morphology and morphometric measurements of eight anuran species which included Asian Common Toad (*Duttaphrynus melanostictus*), Indus Valley Toad (*Duttaphrynus stomaticus*), Ant Frog (*Microhyla ornata*), Common Skittering Frog (*Euphlyctis cyanophlyctis*), Cricket Frog (*Fejervarya spp.*), Hazara Torrent Frog (*Allopaa hazarensis*), Murree Hills Frog (*Nanorana vicina*) and Bull Frog (*Hoplobatrachus tigerinus*). Despite best efforts, tadpoles of Burrowing Frog (*Sphaerotheca breviceps*) and Balloon Frog (*Uperdon systoma*) could not be recorded. Likewise, *Fejervarya* species posed complex identification issues, and the two species (*F. limnocharis* and *F. syhadrensis*) were treated as *F. spp.* Principle Component Analysis produced nine factors, of which first three factors accounted for 80% of the variability in the data. Factors such as elevation, TDS, salinity, conductivity, air temperature and humidity were associated (factor loading > 0.5) with PCA factor 1. Elevation showed high negative correlation ($R^2 > 0.7$) with water temperature and salinity was positively correlated with conductivity and air temperature. Cluster analysis produced two main clusters of sampling sites: Cluster I had sites (1, 2 and 3) which featured sub-tropical pine forest located above 1000 m. elevation with low air temperature and wetlands with low water temperature. The associated anuran species included Murree Hills Frog (*Nanorana vicina*), Hazara Torrent Frog (*Allopaa hazarensis*), Asian Common Toad (*Duttaphrynus melanostictus*), Common Skittering Frog (*Euphlyctis cyanophlyctis*) and Cricket Frog (*Fejervarya spp.*). The cluster II had sites (4 to 11) featuring sub-tropical scrub forest located between 400-650 m. elevation with relatively higher air and water temperature. The associated anuran species included Asian Common Toad (*Duttaphrynus melanostictus*), Indus Valley Toad (*Duttaphrynus stomaticus*), Common Skittering Frog (*Euphlyctis cyanophlyctis*), Cricket Frog (*Fejervarya spp.*) and Bull Frog (*Hoplobatrachus tigerinus*).

CLIMATE CHANGE A THREAT TO AMPHIBIAN POPULATIONS-A REVIEW**Muhammad Saeed*, Muhammad Rais, Ayesha Akram and Waseem Ahmed***Department of Wildlife Management, PMAS Arid Agriculture University Rawalpindi*

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Climate change is a phenomenon of long term changes in statistical distribution of weather patterns. The current global warming trend is an output of human expansion of the greenhouse effect; warming as a result of heat radiation trapped in atmosphere. The major consequences of climate change include global temperature rise, sea level rise, warming oceans, shrinking ice sheets, declining Arctic sea ice, glacial retreat, ocean acidification and decreased snow cover. The earth average temperature has increased by 0.6 °C during past century. Temperatures of Pakistan increased i.e. minimum and maximum temperatures for all season throughout country. It is feared that several species might fail to adapt to or evolve with this rate of increase in temperature and accompanying other changes in environment. About 15-37% of plants and animals species are vulnerable to climate change induced extinction in the next 50 years. There is a growing concern over possible effects of recent climate change, particularly rise in atmospheric temperature, on amphibian populations. Climate change could affect lentic breeding amphibians directly by reducing their survival and fecundity, or indirectly by altering their habitats. Moreover, climate change can impact breeding, foraging and overwintering habitats that are used by amphibians in different life-history stages. The increase of temperature and thermal variation affect breeding phenology, shift in distribution patterns and spread of epidemics. Pakistan is a home of twenty six anurans species of which six are endemic to Pakistan. Northern areas of Pakistan have high degree of endemism of anurans and vulnerable to temperature changes. The data on impacts of climate change on high elevation anuran species of *Allopaa*, *Scutigera* and *Nanorana* are lacking in Pakistan. However, it is feared that these species might face similar consequences as that of anurans found elsewhere in the world.

EVALUATION OF RENAL FUNCTION AND ELECTROLYTE IMBALANCE IN AUTOMOBILE MECHANICS AND PETROL PUMP ATTENDANTS**S. Rabeel, N. Roohi and B. Javed***Department of Zoology, University of the Punjab, Quaid-e-Azam campus, Lahore-54590*

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This study aims to evaluate the renal function in the serum of automobile and petrol pump workers by comparing serum creatinine, uric acid, sodium (Na⁺), potassium (K⁺), bicarbonate (HCO₃⁻) and chloride (Cl⁻) with healthy controls. Of the total 70 male individuals included in this study, 28 were automobile workers 19 were petrol pump workers and the remaining 23 were control. Renal function indices of all the participants were measured by using commercially available diagnostic kits. A non-significant decrease in serum creatinine and uric acid concentrations were observed as compared to control. There was a significant decrease in sodium and chloride concentration and a non-significant increase in potassium level in workers when compared with control. Non-significant decrease of bicarbonate concentration in petrol pump workers and significant increase in automobile workers was found when compared with control. These findings indicate that the chemicals and their metabolites absorbed during occupational exposure might have reacted and interacted with the renal tissues to impair the kidney function.

GONADAL STEROIDS AND THEIR REGULATORY HORMONAL HOMEOSTASIS IN WORKERS OF AUTOMOBILE WORKSHOPS AND PETROL FILLING STATIONS**Bilal Javed and Nabila Roohi***Department of Zoology, University of the Punjab, Quaid-e-Azam campus, Lahore-54590*

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Among different occupations, petrol pump attendants (PPA) and automobile workers (AMW) are most vulnerable people to the occupational toxicity caused by heavy metals or PAHs. Recent studies showed

abnormal production of gonadal and its regulatory hormones. Gonadal and its regulatory hormonal patterns were determined in petrol pump attendants and automobile workers. For this purpose, blood samples of 19 petrol pump attendants and 29 automobile workers were collected from different petrol pumps and automobile workshops, respectively. The blood samples for the control were collected from University of the Punjab, Lahore. Samples were analyzed for determination of serum Estradiol, follicle stimulating hormone, luteinizing hormone and Testosterone. Anthropometric parameters including body weight, body height, body mass index, pulse rate, systolic and diastolic blood pressure were also determined. Testosterone to estradiol ratio (T/E) was also determined. Non-significant variations were observed in the all anthropometric parameters. Mild decrease was observed in estradiol (27.76 ± 2.77 in PPA and 31.15 ± 1.98 AMW), FSH (5.19 ± 0.65 in PPA and 4.80 ± 0.75), LH (3.38 ± 0.41 in PPA and 3.38 ± 0.41 in AMW) and testosterone concentration (4.43 ± 0.52 in PPA and 4.43 ± 0.43 in AMW) in study groups. Whereas, non-significant increase in the (T/E) in petrol pump attendants, and non-significant decrease in the (T/E) were observed in automobile workers. Mild decrease in hormones suggested that exposed males are at the risk of hypogonadotropic hypogonadism.

EVALUATION OF BIOREMEDIATION ACTIVITY OF CHROMIUM-RESISTANT BACTERIA USING POLLUTED WATER SAMPLES

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Chromium is a toxic heavy metal which has several adverse effects on plant growth such as disrupting the structure of cell membrane and degrading photosynthetic pigments etc. These harmful effects of chromium impose stress in plants and retard their growth. This problem can be overcome by using some chemical methods, however, chromium-resistant bacteria can also be used as a bioremediation tool to reduce chromium toxicity. In the present work, *Helianthus annuus* seeds were inoculated with five identified strains of chromium-resistant bacteria belonging to *Halomonas*, *Kushneria*, *Bacillus* and *Arthrobacter* spp. After bacterial treatments, the seeds were sown in pots containing sieved soil and were watered with polluted and non-polluted waters such as chromium-contaminated water obtained from industrial areas, Kasur; chromium-contaminated water containing K_2CrO_4 and non-polluted water without chromium contamination. Pots watered with distilled water were taken as control. Different growth parameters such as root length, shoot length, fresh weight and number of leaves of the experimental and control plants were recorded. It was observed that plants treated with different concentrations of chromium were stunted in growth. However, when they were inoculated with chromium-resistant bacteria, the growth of the plants were improved compared to non-inoculated treatments. Similarly chromium-contaminated water affected the biochemical parameters of the plants adversely. However, bacterial treatments reduced the toxicity of the chromium present in water leading to better development of plants so these strains can be used as bioremediation agent to reduce chromium content in the polluted waters.

COMPARISON OF ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SULFONAMIDE DERIVATIVES

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The antimicrobial activity of various antibiotics has been recognized for many years. However, reducing bacterial susceptibility and increasing *Escherichia coli* (*E. coli*) resistance is reported frequently in recent studies which encourage the synthesis of new drugs to provide effective alternative to physicians. To this end, we synthesized 27 monosulfonamide derivatives and tested their antimicrobial potential against *E. coli*. The results confirmed the successful *E. coli* inhibition by two monosulfonamide derivatives. Furthermore, derivative

synthesis, structure, effect of potent derivatives in combination and mechanism of *E. coli* inhibition will be discussed.

**ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SULFONAMIDE DERIVATIVES AGAINST
ENTEROCOCCUS FAECALIS AND *ENTEROCOCCUS FAECIUM***

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Enterococci are gram positive bacteria that are part of the normal intestinal flora of most humans. In the last two decades, several reports have documented that the two most important species, *Enterococcus faecalis* and *Enterococcus faecium*, are among the leading cause of several human infections, including bacteremia, septicemia, endocarditis, urinary tract infections, wound infections, neonatal sepsis and meningitis. Therefore, synthesizing and developing new drugs is always desirable. To this end, we synthesized 11 sulfonamide derivatives with pyrazolotetrazolo-triazine core and tested their antimicrobial potential against *Enterococcus faecalis* using disc diffusion method. The result confirmed the dose depended inhibition effect of all tested sulfonamide derivatives. Furthermore, derivative synthesis, structure and mechanism of microbial inhibition will be discussed.

**SYNTHESIS OF AMINO-SUBSTITUTED PYRAZOLO TRIAZINES AND
EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES**

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Enterococci are associated with infections of the urinary tract, wound, and, and bloodstream; infective endocarditis; and, rarely, meningitis. Up to 90% of enterococcal infections in humans are caused by *Enterococcus faecalis* (*E. faecalis*). Therefore, developing new strategies against *E. faecalis* is always of interest. To this end, we synthesized 20 Amino-Substituted Pyrazolo Triazine derivatives and tested their antimicrobial potential against *E. faecalis*. The result confirmed their antimicrobial effect in a dose dependent. Furthermore, mechanism of microbial inhibition and comparative drug inhibition will be discussed.

ANTIBACTERIAL ACTIVITY OF SYNTHESIZED MONOSULFONAMIDE DERIVATIVES

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Enterococcus faecalis, the predominant human enterococcus, has been frequently reported to cause oral ailments, such as caries, endodontic infections, periodontitis, peri-implantitis and also implicated in failure of endodontic treatment, due to high resistance to endodontic medicaments. Therefore, synthesizing and developing new drugs is always desirable. To this end, we synthesized 27 monosulfonamide derivatives and tested their antimicrobial potential against *E. faecalis*. The results confirmed the test compounds concentration depended microbial inhibition. Furthermore, mechanism of microbial susceptibility and comparative drug inhibition will be discussed.

ANTI-BACTERIAL ACTIVITY OF SOME SELECTED ANTISEPTIC DRUGS AVAILABLE IN MARKET (PAKISTAN) AGAINST FREQUENTLY ENCOUNTERED BACTERIA

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The in vitro antibacterial activity of four well-known antiseptic drugs available in market was conducted by agar disc diffusion method. Reference bacterial strains; *Enterococcus faecalis* (E. faecalis), *Enterococcus faecium* (E. faecium) and *Klebsiella pneumonia* (K. pneumonia) were treated with discs having 30mg of each drug namely Amoxicillin (AM), Chloramphenicol (CH), Kanamycin (KA) and Nalidixic acid (NA). Antibacterial activity of these drugs was different from each other depending on bacteria type being tested against. *E. faecium* showed susceptibility pattern as KA>AM > CH >NA, *E. faecalis* showed susceptibility pattern as AM> CH>KA>Na, however, *K. Pneumoniae* showed susceptibility pattern as KA>CH> NA>KA. All tested drugs gave satisfactory results. However, further comparative study of selected drug alone and in combination with each other is being conducted and will be discussed.

SOME PRELIMINARY BIOCHEMICAL OBSERVATIONS ON TWO SPECIES OF SEA SNAKES (ELAPIDAE: HYDROPHIINAE) FOUND IN SONMIANI WATERS OF BALUCHISTAN, PAKISTAN

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Pakistani sea waters served as a favored habitat to different species of sea snakes. Most of the previous studies reported 14 species in Pakistani waters from more than 62 (worldwide) reported species of poisonous Sea snakes. The literature review revealed no previous research based on biochemical and molecular work on the sea snakes of Pakistan. Recently, the representatives of two species (*Hydrophis shistosa* and *Hydrophis caeruleus*) of family Hydrophiinae were collected from Sonmiani waters. The average length of *Hydrophis shistosa* was 8 ft and *Hydrophis caeruleus* with average length of 3.5 ft. The biochemical constituents (Protein, Carbohydrate, Lipid and Moisture contents) of body muscles were examined for both the species. The general proteins were estimated by using two different stain (Coomassie brilliant blue and Amido black) through SDS page analysis and the molecular weight fraction were also estimated for the muscle proteins through using known weight of protein markers.

SYNTHESIS AND BIOLOGICAL EVALUATION OF SILVER NANO-ASSEMBLIES

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Nanotechnology has emerged as a novel tool for the modulation of metals into their nano-size and thus changes their physico-chemical and optoelectronic properties. Silver nanomaterials possess great antimicrobial potential and are extensively used in various biomedical applications such as dressings, medical devices and cosmetics. In the present study, three kinds of silver nano-assemblies have been fabricated separately in a one-step reaction. These are designated as 3D-3D, shell-3/1D and 3D-1D nano-architectures. These nano-architectures were synthesized by polyol reduction method in the presence of polyvinyl pyrrolidone (PVP) at 120, 140 and 160°C, respectively. During the experiment, controlled temperature decrease has been carried out which successfully developed 3D-3D, and 3D-1D conjunction. The shell-3/1D nano-assembly was successfully developed at 140 °C by gradual decreasing and subsequently increasing of temperature. The size and morphology of each product was characterized by transmission electron microscope (TEM). Surface plasmon resonance (SPR) behaviour was analyzed by UV- Vis spectroscopy. The antimicrobial activities were also

analyzed for gram positive bacteria like *Enterococcus faecalis* and *Staphylococcus aureus*, and gram negative like *E. coli* 0157:H7 and DH5a by scanned optical density (OD) and Kirby-Bauer process. The OD was measured by taking scanned UV-Vis absorption from 700–300 nm wavelength in LB (Luria-Bertani) broth media. Similarly Kirby-Bauer process was applied over LB agar media. The material was found unique in terms of morphology as well as in displaying its antimicrobial activities both for gram positive and gram negative bacteria.

BACILLUS AMYLOLIQUEFACIENS 6A: A NOVEL KEROSENE OIL DEGRADING BACTERIUM

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Petroleum hydrocarbons pollution is now one of the biggest environmental problems resulting from anthropogenic and/or non-anthropogenic sources and poses a serious threat to human health as these pollutants are known to have carcinogenic and neurotoxic effects. The present investigation is dealing with the use of bacterial strain (*Bacillus amyloliquefaciens*) isolated from kerosene contaminated industrial wastewater and identified biochemically and by ribotyping, to degrade kerosene hydrocarbons from aqueous and soil environments. The bacterial strain possesses kerosene degradation potential and could remove 13%, 39%, 40% and 64% kerosene oil as compared to the microorganisms indigenously present in industrial effluent which could degrade only 14%, 16%, 27% and 28% kerosene oil after 2, 4, 6 and 8 days, respectively. Kerosene biodegradation behaviour was evaluated by studying biodegradation kinetics, half-life and thermodynamics to determine the suitability of the process. Kerosene biodegradation process followed pseudo first order kinetics and found greatly influenced with temperature. Degradation rate constants and overall degradation rate was calculated from line waver burke plots obtained from Michaelis-Menten equation. Moreover, hydrocarbon fractions of kerosene oil before and after bacterial treatment were estimated by GC-MS and thin layer chromatography (TLC). Moreover, enzyme activity of crude extracellular lipase was calculated and relationship between concentration of kerosene oil and enzyme activity (oil degradation) was established by Michaelis-Menten plot. The enzyme followed the single substrate Michaelis-Menten kinetics with V_{max} (maximum rate) 9.251 ($\mu\text{g.ml}^{-1}\text{min}^{-1}$) and K_m (Michaelis constant, substrate affinity) 8.325 $\mu\text{g.ml}^{-1}$ for kerosene oil. Furthermore, hydrocarbon fractions of kerosene oil before and after bacterial treatment were estimated by GC-MS and thin layer chromatography (TLC) which revealed the degradation of many aliphatic and aromatic hydrocarbon constituents of kerosene.

SALINITY TOLERANCE OF SOME COMMERCIALY IMPORTANT FISHES BEING CULTURED IN PAKISTAN

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In the present study, salinity tolerance of some commercially important fishes was investigated in intensive aquaculture system. The juveniles of red tilapia, *Oreochromis* sp ($5 \pm 0.07\text{g}$), Nile tilapia, *Oreochromis niloticus* ($4.5 \pm 0.05\text{g}$), common carp, *Cyprinus carpio* ($6 \pm 0.04\text{g}$), sea bream, *Acanthopagrus berda* ($5.84 \pm 0.02\text{g}$) and Milk fish, *Chanos chanos* ($5.5 \pm 0.03\text{g}$) were randomly distributed into seawater tanks ($60 \times 30 \times 45$ cm each). Ten fish were stocked in each with 2 replications for each treatment to assess the effects of salinity levels (5‰, 10‰, 15‰, 20‰, 25‰, 30‰ and 40‰) on growth performance, feed utilization, body composition and survival rate. Fish were fed with commercial floating pellet (35% protein) at 3% body weight/day for 40, 50 and 60 days, respectively. The important findings of this study are summarized as follows: Red tilapia, Nile tilapia, common carp, sea bream and milkfish showed significantly higher growth ($P < 0.05$) in term

of weight gain, SGR, feed conversion and survival rate from 15‰ to 30‰, 10‰ to 20‰, 5‰ to 15‰, 20‰ to 30‰ and 25‰ to 30‰ salinity, respectively. Feed conversion ratio were found similar in all treatments. Biochemical analysis of fish meat showed that moisture, protein, lipid, ash and crude fiber were not significantly ($P>0.05$) affected by salinity level. Therefore, present study suggests that red tilapia, seabream and milkfish can be cultured up to 30‰, whereas Nile tilapia and common carp can be cultured up to, 20‰ and 15‰ salinity to get maximum growth and survival rate.

REVIEW AND ASSESSMENT OF THE LITERATURE ON THE ORIGIN OF WHALES

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This Poster evaluated the literature regarding the beginning of first whales in the fossil record around 52.5 million years ago (Mya) during the early Eocene in Indo-Pakistan. Our acquaintance of early and middle Eocene whales has increased dramatically during the past three decades to the point where hypotheses of whale origins can be supported with an immense deal of confirmation from paleontology, anatomy, stratigraphy, and molecular biology. Fossils also provide preserved verification of behavior and habitats, allowing the reconstruction of the modes of life of these semi-aquatic animals during their transformation from land to sea mammals. Modern whales originated from earliest whales at or near the Eocene/Oligocene boundary, approximately 33.7 Mya. During the Oligocene, ancient whales coexisted with early baleen whales and early toothed whales. By the last part of the Miocene, most contemporary families had originated, and most ancient forms had gone extinct. This review concluded about the Whale diversity climaxed in the late middle Miocene and fell thereafter toward the Recent, yielding our depauperate modern whale fauna.

A CRITICAL LITERATURE REVIEW AND ASSESSMENT OF CETACEAN MORTALITY IN THE TUNA FISHERIES OF PAKISTAN

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This poster is about review and appraisal of literature on pelagic gillnets used for catching tuna known for high by catch and mortality of cetaceans. Pakistan is one of the few countries somewhere gillnet is being used for catching tuna species, though, no knowledge on the subject of by catch particularly of non-target species for instance cetaceans and turtles was previously collected. In order to collect the information about mortality of cetaceans and other by catch, the attempt was started by WWF-Pakistan under tuna fisheries observers program. Monitoring of landings at Karachi fish harbor was established as well as an observer programme was started. Observers were posted on four tuna gillnet vessels who collected on all sides of the year data of every haul and recorded related information. The data reveals that yellowfin is the main tuna species followed by longtail tuna. Skipjack, kawakawa and frigate tuna are other imperative species. Marked cyclic variation in tuna landings and species composition was noticed. It was also observed that bycatch considerably contribute to the commercial catches consisting mainly billfish (sailfish and marlins), dolphinfish, and sharks. In addition, large number of turtles and dolphins are also caught in the tuna gillnets. Only a few turtles was observed to die if entangled in the gillnets. Olive Ridley turtle was observed to be the most dominating turtle species followed by green turtle. It was interesting that most of the intertwined turtles survive. Throughout this project, an awareness campaign for fishing crew was started to safely release enmeshed turtles which proved successful and now most turtles are careful disentangled from the gill nets and released in the sea. No nesting or stranding of olive Ridley turtle was recorded from Pakistan for the last 11 years, however, a large inhabitants of this turtles was observed in the offshore waters of Pakistan. Population of enmeshed olive Ridley

turtles in the tuna gillnet was estimated to be about 31,000. It is speculated that this population may be nesting in neighboring countries. All enmeshed dolphins were observed to die thus, discarded. It is estimated that about 12,000 dolphins are killed every year in tuna gillnet operation. Indo-Pacific humpback dolphin (*Sousa chinensis*), bottlenose dolphin (*Tursiops aduncus* and *T. truncatus*), spinner dolphin (*Stenella longirostris*), pantropical spotted dolphin (*Stenella attenuata*), long beaked common dolphin (*Delphinus capensis tropicalis*), Risso's dolphin (*Grampus griseus*), striped dolphin (*Stenella coeruleoalba*) and rough tooth dolphin (*Steno bredanensis*) were observed to die in the tuna gillnet operation in coastal and offshore waters of Pakistan. Marked seasonality was observed in the enmeshment of dolphin with maximum mortality in November. No mortality was observed in monsoon months (June and July) as no tuna gill netting is done in these two months. With the exception of mortality of one dwarf sperm whale (*Kogia sima*) no mortality of whales was recorded. Considering exceptionally high mortality of dolphins it is recommended to take appropriate management measures including ban on new entry in tuna gillnet fishing, agreement to UNGARE solutions restricting gillnet length to 2.5 Km, conversion of gillnetting fleet to longlining, declaration of marine protected areas (MPAs), establishment of a regular data base of turtle and cetacean enmeshment and adherence to management measures suggested by IOTC (IOTC).

LITERATURE REVIEW OF SPECIES ASSORTMENT AND DISTRIBUTION PATTERN OF MARINE MAMMALS OF THE PERSIAN GULF AND GULF OF OMAN - IRANIAN WATERS

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This poster literature review confirmed total 98 numbers of marine mammal records from Iranian coastal waters of the Persian Gulf and Gulf of Oman were brought together of which 66 are previously unpublished (new records). Seventy-nine were from the Persian Gulf and 16 from the Gulf of Oman coast. The largest numbers of documentations were from Qeshm Island and Bushehr Provinces. Reports of Indo-Pacific finless porpoise (*Neophocaena phocaenoides*), Indo-Pacific humpback dolphin (*Sousa chinensis*) and Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) were by far the most abundant probably reflecting their inshore distribution and local abundance. Other species recorded are common dolphin (*Delphinus capensis tropicalis*), rough-toothed dolphin (*Steno bredanensis*), Risso's dolphin (*Grampus griseus*), false killer whale (*Pseudorca crassidens*), and dugong (*Dugong dugon*). Evidence of 22 Mysticetes were obtained, eight of which were tentatively identified as Bryde's whales (*Balaenoptera edeni*), three as fin whales (*Balaenoptera physalus*) and three as Humpback whales (*Megaptera novaeangliae*). The main hazard to marine mammals in Iran is likely to be incidental capture in fishing gear. Six by caught Indo-Pacific finless porpoises were recorded and this species may be particularly vulnerable to incidental mortality in gillnets. This poster literature review of marine mammal proposed that research, conservation and management of marine mammal should be practiced through small projects in Iran.

LITERATURE REVIEW ON OCCURRENCE OF WHALES AND DOLPHINS IN PAKISTAN WITH REFERENCE TO FISHERS' ACQUAINTANCE

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This review literature provides use full information regarding the Cetacean Conservation Pakistan. This project started in 2004 with a view to: (1) undertaking quantitative surveys to conclude the variety and abundance of species present; (2) working with local fisher communities to gather confined information and

support community awareness; and (3) promotion of a marine cetacean protection rules and policies. The study of boat-based assessments for live animals and coast assessments for beach cast specimens has verified the incidence of twelve species of whale and dolphin. Among these bottlenose dolphins (*Tursiops* sp.) occur both inshore all along the coasts of Sindh and Balochistan, and offshore in parts of Balochistan; these two populations potential symbolizing different sub-species. Indo-Pacific humpback dolphins (*Sousa chinensis*) are widespread inshore approximately the mouth of the Indus Delta and in large sheltered bays in Balochistan, where Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) also occur. Spinner dolphins (*Stenella longirostris*) were observed in very large schools (up to 2,000) around the shelf edge in eastern Balochistan, as were Risso's dolphins (*Grampus griseus*) in smaller numbers. Common dolphins (*Delphinus capensis tropicalis*) were recorded even further offshore. There were two sightings of humpback whales (*Megaptera novaeangliae*), and one of a killer whale (*Orcinus orca*). Bryde's whales (*Balaenoptera edeni*), sperm whales (*Physeter macrocephalus*) and Cuvier's beaked whales (*Ziphius cavirostris*) were documented only during beach surveys, while skeletal remains in institutions also supported the incidence of blue whales (*Balaenoptera musculus*). Work with local fisher communities supported this picture of species distribution and provided information on threats to local cetaceans. These are principally infrequent entanglement in fishing gear and opportunistic exploitation for use as food, as bait, as medicine or for other purposes. The study incorporated policy enlargement and the preparation of a marine cetacean biodiversity accomplishment plan that included the listing of species in provincial conservation legislation, the designation of a marine protected area in Baluchistan, the establishment of a national whale and dolphin conservation society, and trials of whale and dolphin watching as a means of raising public awareness and providing another economic value.

RECORDS OF WILDLIFE HUNTING FROM AZAD JAMMU AND KASHMIR

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Hunting is a prehistoric practice for food, recreation and revenue generation. However, it poses a serious threat when unregulated to the survival of species especially those which are at the verge of extinction. In the current study, we collected hunting records for wildlife species along with major causes of mortality from different area of Azad Jammu and Kashmir from January 2015 to December 2017. The area was thoroughly searched out to recover dead animals. Moreover, the hunters/local community were interrogated (questionnaire survey) to determine the motive of poaching. The results showed records of numerous wild animals (N=37) killed by local community. Despite protected status, the killed mammals belonged to categories of Critically Endangered (*Pantherapardus*), Near Threatened (*Viverricula indica*, *Canisaureus*, *Hystrix indica* and *Vulpesvulpesmontana*) and Vulnerable (*Hylopetesfimbriatus*) species. The important birds include Critically endangered (*Falco peregrines*), Endangered (*Lophuraleucomelanos*, *Gyps fulvus*), Vulnerable (*Catreuswallichii*, *Pucrasiamacrolopha*, *Tragopanmelanocephalus*, *Streptopeliaturtur*) and Near Threatened species (*Lophophorusimpe janus*). It was found that mammals were primarily killed due to human wildlife conflict (HWC) (44%) followed by recreation (25.75%), trade (19.25%) and accidental killing (11%). Whereas, the foremost hunting reasons for birds are recreation (68.67%) and food (19.67%). Birds are also being used as an important trade commodity (10.56%). The HWC and accidental killing were underlying factors of killing of reptiles (n=8) and amphibians (n=3) respectively. The different techniques employed in animal catching/killing included shooting with guns (>70% for mammals and birds) and use of stick and stone (>50% for reptiles). The reasons for killing differed among mammals, birds, amphibians and reptiles (p<0.005) except for trade (p=0.354). Methods of killing also differed greatly among species (p< 0.001). The findings of the current study highlight the diversity of wildlife species in the study area. Moreover, the hunting/killing of the species brings attention towards the conservation of vital species.

EFFECT OF BUTANOL FRACTION OF *CISSUS QUADRANGULARIS* ON GROWTH PARAMETERS AND PROLIFERATION OF MOUSE PRE-ADIPOCYTE CELL LINE 3T3-L1.**Rabail Hassan Toor, Usama Shameel and Abdul Rauf Shakoori****School of Biological Sciences, University of the Punjab, Lahore*

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Obesity has attained epidemic proportions in both developing and developed countries. It is also an important risk factor of various diseases i.e. diabetes, cardiovascular diseases, dyslipidemia, and hypertension. *Cissus quadrangularis* (CQ) is a medicinal herb that has been reported in ancient Ayurveda literature for its bone healing properties. Beside bone healing, it has also been reported to be effective in management of obesity and metabolic syndrome. The present study explores the effect of Butanol fraction of *Cissus quadrangularis* (CQ-B) on the mouse pre-adipocyte cell line 3T3-L1. The non-toxic concentrations of CQ-B determined by cytotoxicity assay. Growth curve parameters and effect of non-toxic concentrations on cell proliferation were also studied. Further studies will assess the effect of the non-toxic concentrations of CQ-B on differentiation of 3T3-L1 into adipocytes along with histochemical staining and analysis of gene expression of adipocyte marker genes.

DESCRIPTION OF *SIVALHIPPIUS* (EQUIDAE: PERISSODACTYLA) FROM THE LATE MIOCENE SIWALIKS OF PUNJAB, PAKISTAN**Muhammad Tahir Waseem*, Rana Manzoor Ahmad, Ayesha Iqbal, Muhammad Ameen and Abdul Majid Khan***Department of Zoology, University of the Punjab, Lahore*

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The samples described in this paper have been discovered from Dhok Pathan type locality, district Chakwal, Punjab, Pakistan. The sample comprises of isolated pre-molars and molars along with a complete mandible with preserved molars and pre-molars. On the basis of the morphometric comparison of our sample with previously known Hipparion data from the Siwaliks, the specimens were ascribed to the genus *Sivalhippus* and species *S. theobaldi*. The diagnostic features of the *Sivalhippus theobaldi* are large and rectangular molars, increased hypsodonty, compressed and pillar like protocone and simple enamel bordering. The comparison of our sample with living Equid species indicated some similarities in dimensions of molars and pre-molars but differ in having complex enamel bordering which indicates a slightly different feeding niche. The dental morphology indicates that the *Sivalhippus theobaldi* was a dominant grazer which existed in mosaic of woodland and grassland in the late Miocene of the Siwaliks of Pakistan.

INVESTIGATION OF CHOLINERGIC ENZYMES IN HEROIN ADDICTION**Mahwish Iqbal, Arooj Tanveer, Arooj Anwar, Sadaf Munir and Syed M Nurulain***Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan*

Addiction of drug is a chronic relapsing disorder. The development of addiction effect various neurobiological processes which include both drug-use and drug use-dependence. According to the United Nation Office of Drug and Crime (UNODC) report 2015, worldwide incidence of drug dependence has increased more than 29 million in 2014 from 27 million in previous years and heroin is found to be leading addictive drug. Cholinergic enzymes i.e. acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) has been reported to play role in addiction physiology but scarcely investigated. The present study was designed to investigate the status of cholinergic enzymes in heroin addicted individuals in comparison to non-addicted healthy subjects. Ethical approval was taken from departmental ethical committee. Prior consent was obtained from participants of the study. Spectrophotometric measurement of enzymes was based on Ellman's method. The results revealed an increase level of AChE and BChE in heroin addicted individuals than healthy non-

addicted subjects. The increase was statistically significant. It is concluded that cholinergic enzymes have physiological roles in heroin addiction. Further studies is recommended to find a cholinergic enzyme based therapy for heroin addiction.

NATIONAL CULTURE COLLECTION ACTIVITIES OF PARC, PAKISTAN AND THE DESCRIPTION OF SEVERAL NOVEL SPECIES OF BACTERIA FROM PAKISTANI ECOLOGY

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The importance of microbes in biotechnological, agricultural and industrial applications has pushed many Institutes to collect the indigenous beneficial microbes from various ecological zones of Pakistan. Among these, the most common examples are the strains with plant growth promoting activity to be used as biofertilizer; strains for bio-remediation of heavy-metals polluted soils/water systems; pathogenic strains of bacterial blight from rice ecology and citrus canker; and other extremophilic (i.e. NaCl tolerant) strains for biotechnology. However, very few of these microbes have been truly identified at species level based upon 16S rRNA gene sequence. Most of the strains collected by various labs were either stocked in glycerol as un-identified strains or abolished with time due to contamination or loss of interest of researchers at end of the project. In spite of untiring efforts by researchers at various Pakistani Institutes, there is rare example of new species of bacteria published from the rich ecology of Pakistan since long. There was a big challenge to validate any novel species from Pakistani ecology because the systematic efforts have not been made to recognize the strains at national and/or international level based upon scientific background and molecular tagging (gene sequence). Major reasons include lack of specialized equipment's for chemotaxonomic analyses as well as expertise required for validation of any novel species. In Pakistan, long term preservation of microbes is a neglected subject. Recently, Pakistan Agricultural Research Council (PARC) took an initiative to start working on identification and preservation of economically important bacterial strains from Pakistani ecology. In this regard, PARC established microbial bio-resources repository: National Culture Collection of Pakistan (NCCP) for preservation of this bio-asset of Pakistan. During our studies for collection and preservation of indigenous beneficial strains from Pakistani ecology, many promising novel candidate strains were identified based upon 16S rRNA gene sequencing, which can be delineated as novel species. However, many chemotaxonomic experiments need specialized equipment. Unfortunately, such modern techniques/equipments were not present in any lab in Pakistan. Under the scenario, collaboration linkages were developed with CAS, China, JCM, Riken, Japan and KCTC, KRIBB, Korea to delineate our candidate strains as novel species. It is first time in the history of Pakistan, that a productive collaboration made it possible to validate the following several novel species of bacteria from Pakistani ecology:

- *Cellulomonas pakistanensis* sp. nov., (validated – 2014);
- *Sphingobacterium pakistanensis* sp. nov., (validated – 2014);
- *Bacillus pakistanensis* sp. nov., (validated – 2014);
- *Lysinibacillus pakistanensis* sp. nov., (validated – 2014);
- *Lysinibacillus composti* sp. nov., (validated – 2014),
- *Deinococcus citri* sp. nov., (validated – 2014);
- *Acinetobacter pakistanensis* sp. nov., (validation – 2014);
- *Alcaligenes pakistanensis* sp. nov., (validation – 2015);
- *Bacillus malikii* sp. nov. (validated – 2015);
- *Bacillus boracitolerans* sp. nov., (in process of publication - 2014);
- *Kushneria pakistanensis* sp. nov., (validated – 2015);

- *Nocardioides pakistanensis* sp. nov., (Validated – 2016)
- *Microvirga pakistanensis* sp. nov., (published – 2016)
- *Streptomyces caldifontis* sp. nov., (validated – 2017)
- *Thermus caldifontis* sp. nov., (published – 2017)
- *Nocardioides thalensis* sp. nov., (published – 2017)

Our goal is to collect many more strains from various Institutes for identification and preservation in NCCP. Since, identification and systematics of bacteria have not been extensively included in the main applied microbiological research work in Pakistan, therefore, this rich bio-asset offers an opportunity to explore many novel species of bacteria by identification, poly-phasic characterization and thus to preserve in National Culture Collection of Pakistan (NCCP), which is the ultimate goal of PARC project.

ALCALIGENES PAKISTANENSIS SP. NOV., A HEAVY-METAL TOLERANT NOVEL PLANT GROWTH PROMOTING BACTERIUM, ISOLATED FROM INDUSTRIAL EFFLUENT IN PAKISTAN

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Soil pollution with heavy metals have adverse effect on microbial community and soil health. The present study was conducted to isolate heavy metal tolerant bacterial strains and to elucidate their potential usage for soil bioremediation and in agriculture. Two strains, NCCP-650^T and NCCP-667, were isolated from industrial effluent and their taxonomic positions were investigated using a polyphasic taxonomic approach. The results showed that these strains tolerated concentration up to Cr 1500 ppm, As 3000 ppm, Pb 2100 ppm and Cu 1800 ppm. The molecular characterization for *nifH* and *acdS* gene(s) showed that these strains were found to contain both genes in their genome. Five strains (including NCCP-650^T and some other such as NCCP-644, NCCP-614, and NCCP-602) were further investigated for plant growth promotion activity in *Brassica napus* under axenic condition when irrigated with water containing 50 ppm of each metal separately. The results showed that strain NCCP-650 proved to be the best for increase in growth of *Brassica* plants due to presence of both *nifH* and *acdS* genes. These strains were found to be Gram-stain negative, strictly aerobic, motile short rods. Cells can grow at a temperature range of 10–37°C (optimal 25–33°C), pH range of 5.5–10.0 (optimal 6.5–7.5) and can tolerate 0–7 % NaCl (w/v) (optimum 0–1%) in tryptic soya (Difco) agar medium. Sequencing of 16S rRNA gene and two housekeeping genes, *gyrB* and *nirK* of the isolated strains revealed that both strains belong to the *Betaproteobacteria* showing highest sequence similarities with members of genus *Alcaligenes*. DNA–DNA hybridizations between the two strains and with closely related type strains of species of the genus *Alcaligenes* confirmed that both isolates belong to a single novel species within the genus *Alcaligenes*. The chemotaxonomic data [major quinones as Q-8; predominant cellular fatty acids as summed features 3 (C_{16:1} ω7c/ iso-C_{15:0}2OH) and C_{16:0} followed by Summed features 2 (iso-C_{16:1} I/ C_{14:0} 3OH), C_{17:0} Cyclo and C_{18:1} ω7c; major polar lipids as diphosphatidyl glycerol, phosphatidylglycerol, phosphatidylethanolamine and one unidentified aminolipid] also supported the affiliation of the isolated strains with the genus *Alcaligenes*. On the basis of phylogenetic analyses, physiological, biochemical characteristics and DNA–DNA hybridization, the isolated strains can be differentiated from established *Alcaligenes* species and thus representing a novel species, for which the name *Alcaligenes pakistanensis* sp. nov. is proposed with the type strain NCCP-650^T (= LMG 28368^T = KCTC42083^T = JCM 30216^T). Our results also indicated that these heavy-metals tolerant strains may have the potential for plant growth promotion and can be used as bioinoculants (biofertilizer) in agriculture.

MUTATIONAL SCREENING OF HEPcidIN GENE IN HEREDITARY HEMOCHROMATOSIS WITH H63D HOMOZYGOUS/HETEROZYGOUS PATIENTS IN PAKISTAN

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Hereditary hemochromatosis is an autosomal recessive ailment characterized by p.H63D and pC282Y mutations in HFE gene. Its prevalence is restricted, signifying the probability of other genetic modulators being entangled in iron regulation. In this work, we screened for hepcidin gene polymorphisms in 100 β -thalassemic (major) patients, exhibiting H63D homozygous and heterozygous traits along with intricate clinical and hematological afflictions by PCR-RFLP. Our results manifest a A>G and G>A polymorphisms at hepcidin splicing acceptor site associate in 4 H63D heterozygous patient exhibiting elevated serum ferritin level along with hepatomegaly, splenomegaly and HCV reactivity. Moreover, it was perceived that this polymorphism will disrupt the posttranscriptional activity of the hepcidin pre mRNA and correspondingly lead L98V and S30C modification in one of the patient which has a stabilized effect of -0.01 and -0.27 kcal/mol on protein structure. Our statistics recommend that analysis for these polymorphisms could be of great concern in order to elucidate the decrease hepcidin expression in hemochromatosis patients. Hence, our results suggest that a mutation in the hepcidin splicing enhancer site endorse deregulation of iron metabolism.

THE EXPERIENCE OF THE P.V. ELECTRIFICATION AND THE SOLAR ENERGY OF SOME LOCALITIES IN THE SOUTH OF ALGERIA

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Algeria, located in North Africa, with an area of more than 2.300.000 Km², 4/5 of it are arid and semiarid zones. This particular situation makes it very costly to extend the conventional energy distribution grid, mainly the villages in Sahara are scattered. Further, the average daily total horizontal radiation calculated over the year is about 6 Kwh/m² for over 3000 hours of sunshine duration. Taking in to consideration this huge renewable energy potential, for this reason, an Algerian governmental solar energy program is retained and a tremendous effort has been done to promote the use of the solar energy. The P.V. electrification of many localities in the south of Algeria is installed. For example: Tin Zaouatine, Ain Guezzam, Bordj Baji Mokhtar, Ain Belbel, Aouelef, Matriouane, etc. For example, in a remote saharian village in the south of Algeria, a 6,7 Kwp PV array was installed for the electrification for streets and indoor lighting. The solar energy it is also used in the pumping system for the drinking water and for some needs of the agriculture irrigation. The full solar energy solution has been chosen for the electrification of these isolated regions and to promote the living conditions, such as lighting and other applications. At present the photovoltaic system is running perfectly.

THE STUDY OF THE WATER POLLUTION IN THE WEST OF ALGERIA

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One of the objectives of a modern society is the improvement of the living conditions of the Man. If one goes up the course of the centuries, the history teaches us that the water pollution accompanied all civilizations and that it was always a concern for the legislator until the beginning of the century. Nature, thanks to its intrinsic capacities of regeneration managed to overcome the misdeeds of the man. The pollution developed because of the concentration of the population and the development of her industrial activities. Another time,

the pollution water was primarily of organic nature biodegradable never not exceeding the purifying capacities of the rivers, but the industrial evolution contributed to the marketing. Molecules polluting, that micro the organizations cannot always metabolize and of which some constitute a danger to the Man and his environment. An awakening of an alarming situation as regards quality of water dates from the Seventies. All the types of water were concerned, the subsoil waters, until there with the shelter, are also reached and from now on, one does not cease speaking about nitrates, micro mineral and organic pollutants. Vis-a-vis with this very critical situation at the same time for the health of the man and the environment, the resources water; a vast program of prevention and fight launched out to Algeria in the Seventies/80 and which aimed, the construction of several stations of purification in the most important cities, generally of a size higher than 20. 10' living Following an assessment of this effort of investment established in 1989, it appears clearly that objectif stations of purification was unfortunately not reached but on the contrary the situations developed well, then it appears very important to us to draw up through this modest work, a point of topicality on the state of the quality of water in our area of West of Algeria, with an analysis of the causes of the pollution and the search for the solutions to decrease by its impact.

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