

**PROCEEDINGS**  
**OF**  
**PAKISTAN CONGRESS OF ZOOLOGY**

**Volume 34, 2014**

*All the papers in this Proceedings were refereed  
by experts in respective disciplines*



**THIRTY FOURTH PAKISTAN CONGRESS OF ZOOLOGY**

*held under auspices of*

**THE ZOOLOGICAL SOCIETY OF PAKISTAN**

*at*

**BAHAUDDIN ZAKARIYA UNIVERSITY, MULTAN**

**FEBRUARY 25-27, 2014**

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**Website: [www.zsp.com.pk](http://www.zsp.com.pk)**

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## PROCEEDINGS OF THE CONGRESS

### *Editors*

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## **ACKNOWLEDGMENTS**

Bahauddin Zakariay University, Multan hosted the 34<sup>th</sup> Pakistan Congress of Zoology (International).

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

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# 34<sup>th</sup> PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

## BAHAUDDIN ZAKARIYA UNIVERSITY, MULTAN

February 25 – 27, 2014

### PROGRAMME

#### TUESDAY, FEBRUARY 25, 2014

08:00 AM	Registration
09:00 AM	Inauguration: Recitation from the Holy Quran
09:05 AM	Welcome Address
09:10 AM	Address by Secretary General, Zoological Society of Pakistan
09:20 AM	Address by the President, Zoological Society of Pakistan
09:30 AM	Address by the Vice-Chancellor, BZU
09:40 AM	Distribution of Medals and Awards
10:10 AM	Address by the Chief Guest
10:35 AM	Vote of Thanks
10:40 AM	Refreshment

#### JOINT SESSION I: Plenary Lectures

**Chairperson:** Prof. Dr. M.H. Qazi

**Co-chairperson:** Prof. Dr. Shamsuddin Sheikh

- Speakers: 1. **Dr. Syed Akhter Ali**  
*Department of Cancer Biology, College of Medicine, Mayo Clinic, Jacksonville, Florida, USA*  
**Molecular Regulatory Factors and Cancer Stem Cell Fate Decisions.**
2. **Prof. Dr. Aleem Ahmad Khan**  
*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan*  
**Nitrate Contamination in Drinking Waters of Punjab: A Potential Cause of Early Childhood Mortality in Pakistan.**
2. **Dr. Jirjis Haider**  
*16 L-1, Valencia Town, Lahore*  
**Quran on Human DNA and Its Origin**
- 01:00 PM Lunch and Prayer

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

	Chairperson:	Dr. M. Afzal Ghauri
	Co-chairperson:	Dr. Naveed Shahzad
02:15 AM	Paper reading	
04:30 PM	Tea Time	

**SESSION II**

	Chairperson:	Prof. Dr. Syed Shahid Ali
	Co-chairperson:	Dr. Farah Rauf Shakoori
05:00 PM	Paper reading	
06:25 PM	Prayer	

**SESSION III**

	Chairperson:	Prof. Dr. Qazi Javed Iqbal
	Co-chairperson:	Dr. Bushra Muneer
06:40 PM	Paper reading	
07:00 PM	Executive Council Meeting	
08:30 PM	Dinner	

**HALL – 2****SECTION II: PEST AND PEST CONTROL****SESSION I**

	Chairperson:	Prof. Dr. Mushtaq A. Saleem
	Co-chairperson:	Dr. Riffat Sultana
02:15 PM	Paper reading	
04:30 PM	Tea Time	



**SESSION II**

	Chairperson:	Prof. Dr. Nasreen Memon
	Co-chairperson:	Dr. Abid Fareed
05:00 PM	Paper reading	
06:30 PM	Prayer	

**SESSION III**

	Chairperson:	Dr. Muhammad Hamed
	Co-chairperson:	Dr. Shafqat Saeed
06:40 PM	Paper reading	
07:00 PM	Executive Council Meeting	
08:00 PM	Dinner	

**HALL – 3****SECTION IV: PARASITOLOGY****SESSION I**

	Chairperson:	Prof. Dr. Syed Akram Shah
	Co-chairperson:	Dr. Noor-un-Nisa
02:15 AM	Paper reading	
04:30 PM	Tea Time	

**SESSION II**

	Chairperson:	Prof. Dr. Fatima Mujib Bilqees
	Co-chairperson:	Prof. Dr. Asmatullah Kakar
05:00 PM	Paper reading	
06:30 PM	Prayer	

**SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY****SESSION I**

	Chairperson:	Prof. Dr. Muhammad Akhtar
	Co-chairperson:	Dr. Zaigham Hasan
06:40 PM	Paper reading	
08:00 PM	Dinner	

## WEDNESDAY, FEBRUARY 26, 2014

### JOINT SESSION II: (Plenary Lectures)

**Chairman:** Prof. Dr. A.R. Shakoori

**Co-chairman:** Prof. Dr. Imtiaz Ahmad

- 09:00 AM 1. **Mr. Abdul Aziz Khan**  
*Islamabad*  
**Wondrous World of Earthworm**
2. **Dr. Muhammad Afzal Ghouri**  
*Head, Industrial Biotechnology Division, NIBGE, Faisalabad*  
**Status of Coal Biotechnology in Pakistan**
3. **Dr. Ejaz Masood**  
*Head Oncology Department, Nishtar Hospital, Multan*  
**Breast Cancer: A Common Problem Among Women.**

### HALL – 1

#### SECTION I: CELL BIOLOGY, BIOCHEMISTRY, GENETICS, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

#### SESSION IV

	Chairperson:	Prof. Dr. Muhammad Ali
	Co-chairperson:	Dr. Abdul Rehman
10:30 AM	Paper reading	

#### SESSION V

	Chairperson:	Prof. Dr. Nusrat Jahan
	Co-chairperson:	Dr. Samina Qamer
12:00 AM	Paper reading	
01:30 PM	Lunch and Prayer	

**SESSION VI**

	Chairperson:	Dr. Shahid Nadeem
	Co-chairperson:	Dr. Amtul Jamil Sami
02:00 PM	Paper reading	
04:30 PM	Tea Break	

**SESSION VII**

	Chairperson:	Prof. Dr. Rubina Mushtaq
	Co-chairperson:	Dr. Soumble Zulfiqar
05:00 PM	Paper reading	
08:00 PM	General Body Meeting	
08:30 PM	Dinner	

**HALL – 2****SECTION III: ENTOMOLOGY****SESSION I**

	Chairperson:	Prof. Dr. Imtiaz Ahmad
	Co-chairperson:	Dr. Amjad Farooq
10:30 AM	Paper reading	

**SESSION II**

	Chairperson:	Prof. Dr. M. Saeed Wagan
	Co-chairperson:	Dr. Khawaja Raees Ahmad
12:00 AM	Paper reading	
01:30 PM	Lunch and Prayer	

**SESSION VII**

	Chairperson:	Prof. Dr. M. Ather Rafi
	Co-chairperson:	Dr. Syeda Azra Qamar
02:00 PM	Paper reading	
04:30 PM	Tea Break	

## **SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY**

### **SESSION V**

	Chairperson:	Dr. Muhammad Ashraf
	Co-chairperson:	Dr. Abdul Majid Khan
05:00 PM	Paper reading	
08:00 PM	General Body Meeting	
08:30 PM	Dinner	

### **HALL – 3**

## **SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY**

### **SESSION II**

	Chairperson:	Prof. Dr. Abdul Aleem Ahmed Khan
	Co-chairperson:	Dr. Salma Sultana
10:30 AM	Paper reading	

### **SESSION III**

	Chairperson:	Prof. Dr. Ali Muhammad Yousafzai
	Co-chairperson:	Dr. Wazir Ali Baloch
12:00 PM	Paper reading	
01:30 PM	Lunch Break and Prayer Break (Zuhar)	

### **SESSION IV**

	Chairperson:	Prof. Dr. Javed Ahmad
	Co-chairperson:	Dr. Sajid Mahmood
20:00 AM	Paper reading	
04:30 PM	Tea Break	

### **SESSION VI**

	Chairperson:	Prof. Dr. Quddusi B. Kazmi
	Co-chairperson:	Prof. Dr. A.R. Abbasi
05:00 PM	Paper reading	
08:00 PM	General Body Meeting	
08:30 PM	Dinner	

## THURSDAY, FEBRUARY 27, 2014

### JOINT SESSION III: (Plenary Lectures)

**Chairman:** Dr. M. Afzal Kazmi

**Co-chairman:** Abdul Aziz Khan

- 09:00 AM 1. **Dr. Muhammad Mumtaz Malik**  
*Department of Forestry and Wildlife Management, University of Haripur, KPK*  
**Community Participation: A Success Story of Wildlife Conservation in KPK**
2. **Prof. Dr. Muhammad Saeed Wagan**  
*Department of Zoology, University of Sindh, Jamshoro*  
**Biodiversity and Distribution of Orthoptera Insect of Pakistan**
3. **Prof. Dr. Muhammad Naeem Khan**  
*Department of Zoology, University of the Punjab, Lahore*  
**Review of Fisheries Development in Pakistan: Vision 2020**

### HALL – 1

#### SECTION I: CELL BIOLOGY, BIOCHEMISTRY, GENETICS, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

##### SESSION VIII

	Chairperson:	Prof. Dr. Zahida Tasawar Khan
	Co-chairperson:	Dr. Zulfiqar Ali Saqib
10:30 AM	Paper reading	
11:00 AM	Tea Break	

##### SESSION IX

	Chairperson:	Prof. Dr. Shamim Akhtar
	Co-chairperson:	Prof. Dr. Sumaira Rasool
12:00 PM	Paper reading	
01:00 PM	Lunch Break	

**HALL – 2****SECTION III: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER  
BIOLOGY, MARINE BIOLOGY****SESSION VII**

	Chairperson:	Prof. Dr. Pirzada Jamal A. Siddiqui
	Co-chairperson:	Dr. Noor-us-Saher
10:30 AM	Paper reading	
11:45 AM	Tea Break	

**SESSION IX**

	Chairperson:	Prof. Dr. N.T. Narejo
	Co-chairperson:	Dr. Zafar Ibal
12:00 PM	Paper reading	
01:30 PM	Dinner	

**HALL – 3****SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER  
BIOLOGY, MARINE BIOLOGY****SESSION VIII**

	Chairperson:	Prof. Dr. Iftikhar Hussain
	Co-chairperson:	Dr. Mukhtar Ahmad Mehar
10:30 AM	Paper reading	
11:45 AM	Tea Break	
03:00 PM	Concluding Ceremony	
03:00 PM	Recitation	
03:05 PM	Congress Report by President ZSP	
03:15 PM	Award Ceremony	
03:20 PM	Concluding Remarks by the Chief Guest	
03:25 PM	Vote of Thanks	
03:30 PM	Refreshments	

## MEMBERS OF THE CONGRESS

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 Shehzadi, A.  
 Sohail, A.  
 Tahir, H.  
 Taj, S.  
 Tariq, M.  
 Tariq, T.  
 Zafar, M.  
 Zahid, S.

#### **MULTAN**

Ali, F.  
 Alvi, Q.U.A.  
 Ayaz, M.M. (Dr.)  
 Bibi, F. (Dr.)  
 Haq, M.R.U.  
 Ishtiaq, A.

Nasreen, S.  
 Saifullah  
 Siraj, Z.  
 Ullah, A.

#### **MIRPUR KHAS, SINDH**

Ansari, M.A.

#### **MUZAFFARABAD**

Ahmad, B. (Dr.)  
 Akhtar, N.  
 Ali, G. (Dr.)  
 Ali, S. (Dr.)  
 Andleeb, S.  
 Awan, M.S. (Dr.)  
 Bashir, N.  
 Batool, I.  
 Begum, A.  
 Din, N.U.  
 Gul, S.  
 Hussain, B.G.  
 Hussain, S.  
 Idrees, S.  
 Ilyas, R.  
 Jabeen, A.  
 Kazmi, S.E.  
 Khan, M.A.  
 Khatak, A.  
 Khursheed, J.  
 Kiayni, F.  
 Kousar, R.  
 Kousar, S.  
 Latif, N.  
 Latif, Z.  
 Minhas, M.A.  
 Mustafa, R.G.  
 Nayyer, A.Q. (Dr.)  
 Rauf, T.

Saeed, F.  
Shafique, I.  
Shakeel, U.  
Tahir, S.  
Ulfat  
Yasmeen, F.

#### **NAWABSHAH, SINDH**

Nazir, N.  
Zardari, M.H.

#### **OKARA**

Wajid, M.M. (Dr.)

#### **PESHAWAR**

Farid, A. (Dr.)  
Hassan, Z.  
Khan, A.U.  
Khan, G.Z.  
Khan, I. (Dr.)  
Yousafzai, A.M. (Dr.)  
Zahid, M.  
Zeb, A.

#### **QUETTA**

Kakar, A. (Dr.)  
Ullah, K. (Dr.)

#### **RAWLAKOT, AZAD KASHMIR**

Khan, M.R. (Dr.)

#### **RAWALPINDI**

Abbasi, S.  
Akhter, S.  
Akram, A.  
Akrim, F.  
Anjum, M.Z. (Dr.)

Arshad, A.  
Atiq, J.  
Balouch, S.  
Baneen, U.U.  
Begum, R.  
Farooq, M.  
Fatima, H.  
Ghaffar, M.  
Gill, S.  
Goursi, U.H.  
Hussain, R.  
Hussain, U.  
Iqbal, Z.  
Jabeen, M.  
Kawan, A.  
Kayani, S.B.  
Khalil, H.  
Mahmood, T. (Dr.)  
Minhas, F.R.  
Mukhtar, T. (Dr.)  
Nadeem, M.S. (Dr.)  
Nasreen, S.  
Nazir, S.A.  
Puswal, S.M.  
Qasim, S.  
Qurat-ul-Ain  
Sani, K.A.  
Saparas, S.  
Shehzad, F.  
Tabassum, A.  
Tasleem, N.  
Ullah, I.  
Zeenat

#### **RAHIM YAR KHAN**

Adeela  
Afzal, S.  
Ahmad, A.  
Ahsan, A.

Akmal, S.	Mughal, T.
Akram, F.	Munawar, Z.
Akram, M.	Munir, B.
Akram, N.	Nadeem, T. (Prof.)
Ali, A.	Naik, A.
Ali, S.	Nawab, M.
Ameer, M.W.	Nawaz, H.
Amin, A.	Nawaz, S.
Anwar, M.	Nisar, A.
Arshad, A.	Noreen, M.
Ashraf, K.	Nosheen, A.
Ayaz, S.	Prince, M.A.
Bashir, A.	Quratulain
Batool, A.	Quratulain
Batool, I.	Ramzan, T.
Erum, S.	Rauf, S.
Fashia	Riaz, A.
Fatima, H.	Riaz, F.
Fatima, N.	Saba, N.U.
Fatima, R.	Sadia
Habiba, U.E.	Saeed, N.
Hameed, A.	Saeed, S.
Hameed, K.	Safdar, S.
Hussain, S.	Salamat, A.
Idrees, S.	Saleem, I.
Iqbal, A.	Sehar, S.
Iqbal, M.	Shafique, S.
Ishfaq, A.	Shahida
Ismail, T.	Shakeel, S.
Javed, K.	Sobia
Kanwal, F.	Sohail, M.
Kashif, M.	Swaira
Khan, A.	Tahir, A.
Khan, N.	Tahir, F.
Kiran, A.	Tanzeela
Latif, S.	Tariq, M.
Majeed, N.	Tayyaba
Manzoor, F.	Wazir, G.
Misbah	Yasmeen, S.

Younas, H.  
Zahoor, J.  
Zaid, F.  
Zobia

**SARGODHA**

Ahmed, B.  
Bashir, R.  
Nawaz, S.K. (Dr.)  
Rani, A.  
Yousaf, M.

**SWABI, KPK**

Wahab, F. (Dr.)

**TANDOJAM**

Abro, G.H. (Dr.)  
Sahito, H.A.  
Sahito, J.G.M.  
Solangi, B.K.  
Syed, T.S. (Dr.)

**U.S.A.**

Ali, S.A. (Dr.)



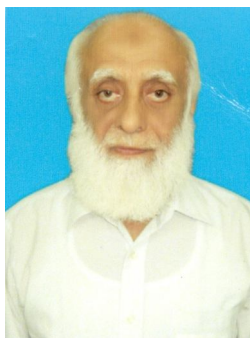


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## CITATIONS

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### RECIPIENT OF LIFE TIME ACHIEVEMENT AWARD 2014



**Prof. Dr. Abdul Qadir Ansari, Sitara-i-Imtiaz**  
*Rector, Al Khair University, Islamabad*

Prof. Dr. Abdul Qadir Ansari was born on 04<sup>th</sup> January 1940 in Hyderabad, Sindh. He received his early education from schools/colleges and University of Sindh, Hyderabad. He did M.Sc. in first class (Gold Medal) from University of Sindh, Jamshoro in 1963 and was appointed as Lecturer in Zoology by the University of Sindh, Jamshoro in 1963. He proceeded to UK for higher studies in 1966 and obtained his Ph.D. in Physiology and Biochemistry in 1969 from the University of Southampton, UK.

He remained post doctoral research fellow from 1969 to 1970 in the University of Southampton, UK, and then senior teaching fellow in the University of Southampton, UK from 1974 to 1975.

He was appointed as Professor of Physiology and Biochemistry by the University of Sindh, Jamshoro from 1973 to 1976. Prof. Ansari was elevated to the position of Founder Vice-Chancellor of Sindh Agriculture University on 1977, where he served till 1988. He was appointed Vice-Chancellor University of Sindh, Jamshoro from 1988 to 1989. He was appointed as Chairman of University Grant Commission from 1989 to 1991. After that, he was appointed Chairman, Pakistan Council for Scientific & Industrial Research (PCSIR) Islamabad from 1992 to 1998. After that, he served as

Chairman, Literacy & Mass Commission Islamabad, from 2000 to 2001. He was appointed as Advisor (Academics) Al-Khair University (AJK) on 2001 to 2004. On 1<sup>st</sup> October 2004 he was appointed as Rector of the Al-Khair University (AJK) where he is still working.

Dr. A.Q. Ansari has 26 National and International publications to his credit. He is Author of the first English to Sindhi directory for Sciences and written three booklets.

In recognition of his contribution towards the scientific research in the field of science, he was awarded Sitara-i-Imtiaz by the President of Islamic Republic of Pakistan on 23<sup>rd</sup> March 1990.

**RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2014**



**Prof. Dr. Muhammad Sharif Khan**

*Ex-Chairman, Department of Zoology, T.I. College, Rabwah*

Prof. Dr. Muhammad Sharif Khan was born in 1939. He obtained B.Sc. degree from University of the Punjab in 1960 and M.Sc. in Zoology from Punjab University, Lahore in 1963. He was awarded Sir William Roberts Gold Medal for standing first in the M.Sc. final examination.

He started his carrier from Talimul Islam College, Rabwah as Lecturer in 1963 and retired from that college in 1999, after 36 years of service as Associate Professor of Zoology. During his stay in that small town, he acclaimed international fame. He proved himself as the lone herpetologist of Pakistan.

Dr. Khan started working on Amphibians and Reptiles (herps) of Pakistan when the knowledge about herps in Pakistan was about nil. When his first research article was published in 1965 in Biologia, Lahore which was based on his M.Sc. thesis "Development of *Bufo melanostictus*," Dr. Robert Mertens from Germany sent a letter to Dr. Khan correcting the name of the toad as *Bufo stomaticus* on which Dr. Khan and his supervisor had worked for about 2 years. This shows the level of knowledge about amphibians among the Zoologists in Pakistan at that time.

Dr. Khan pioneered the study of herps in Pakistan. Dr. Khan has searched almost every corner of Pakistan for reptiles and amphibians and discovered 34 new species, which included 11 snakes, 15 lizards and 8 amphibians.

Prof. Khan has published more than 250 research papers in different scientific journals of the world. He authored 10 books on amphibians and reptiles of Pakistan in English, Urdu and German languages which included (i) *Amphibians and Reptiles of Pakistan*. Krieger Publishing Company, Melbourne, Florida 32902, USA, 2006 (ii) *Die Schlangen Pakistans*, Bucher-Kreth GmbH, Frankfurt, Germany, 2002, (iii) *The Snakes of Pakistan*, Bucher-Kreth GmbH, Frankfurt, Germany, 2002, (iv) *A field guide to the identification of Herps of Pakistan. Part-I: Amphibia*. Biological Society of Pakistan, Lahore 1987, (v) *A field guide to the identification of Herps of Pakistan. Part-II: Chelonia*, Biological Society of Pakistan, Lahore, 1990, (vii). Venomous terrestrial snakes of Pakistan and snake bite problem, In: *Snakes of medical importance* (Asia-pacific region), pp. 419-446, P. Gopalakrishnacon and L.M. Chou (eds). National University of Singapore 1990, (viii) Endangered species of reptiles of Pakistan and suggested conservation measures, pp. 42-45, In: Handbook published to mark second seminar on "*Nature Conservation and Environmental Protection*," 12 March 1991, Islamabad, Wildlife Conservation Foundation, Islamabad,

His Urdu Books include (i) *Amphibians and reptiles of Pakistan*, Urdu Science Board, 299-Upper Mall, Lahore, 2000 Urdu, (ii) *Snakes of Pakistan*, Urdu Science Board, 299-Upper Mall, Lahore pp. 299, (iii) Amphibians, Lizards, Turtles and Snakes. Chapter 2 In: *Wildlife of Pakistan*, Urdu Science Board, 299-Upper Mall, Lahore 1991, (iv) *Pakistan ki hawamiat: Vol.1 Amphibians*. Nia Zamana Publishers, Lahore, 2011.

Dr. Khan has worked on different research projects with WWF-Pakistan and Pakistan Science Foundation. He has supervised a number of M.Sc theses. After his retirement, he donated all his collection to the Natural History Museum, Government College University, Lahore, which includes a countless number of different species of amphibians and reptiles.

Dr. Khan has trained a number of young scientists which are forwarding his mission. Now Dr. Khan is spending a retired life in USA with his son, where he is as active as before, guiding students from Pakistan, Iran and other countries for identification of the species they are working on and in writing their research papers and theses.

In recognition of his contribution to the field of Herpetology Dr. Khan was awarded the Zoologist of the year award in 2002.

**RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2014**

**Dr. Agha Ikram Mohyuddin**

*Ex-Scientist-in Charge and Director, PARC-IIBC Station,  
International Institute of Biological Control, Rawalpindi*

Dr. Agha Ikram Mohyuddin was born in Jullundur, British India on September 21, 1932. He obtained his B.Sc. degree from University of the Punjab, Lahore in 1953 and M.Sc. Zoology Degree in 1956. He later proceeded to Queen's University, Kingston, Ontario, Canada for his Ph.D. degree

Dr. Mohyuddin was Chief Consultant and Chief Executive, Integrated Pest Management Project, 1995-2014; Principal Investigator and Coordinator of all the IPM Projects in Pakistan, appointed by Govt. of Pakistan, 1993-1994; Scientist-in-Charge and Director, PARC-IIBC Station, International Institute of Biological Control, 1980-1994; Principal Entomologist IIBC, 1960-1979; Lecturer, Zoology Department, Govt. College, Lahore 1956-1960; Demonstrator, University of the Punjab, Lahore, 1953-1956; Adviser on Integrated Pest Management (IPM) of Sugarcane Pests, Gunung Madu Plantations Bandar Lampung, Sumatra, Indonesia 1982-1995; Consultant, Malakand Fruit and Vegetable Development Project, a Joint Pakistan/Swiss Government Venture, 1989-1990; Consultant, USAID Funded Project on IPM of Horticultural Crops in Sri Lanka as Staff of Oregon State University/Development Alternative Inc., MD, USA, 1990-1992.

Dr. Mohyuddin received Presidential Award Pride of Performance, 1996; Ismail H. Zakria Gold Medal by Pakistan Society of Sugar Technologists, 1997; Open Gold Medal, Pakistan Academy of Sciences, 1990; Dr. Borlaug Medal, Govt. of Pakistan, 1998; Ghulam Muhammad A. Fecto Gold Medal, 1986-1983.

Dr. Mohyuddin was Member, FAO/UNEP Panel of Experts on IPM 1982-1986; Member, Panel of Experts, University of Agriculture, Faisalabad Pakistan; Member Advisory Committee, Museum of Natural History, Islamabad, 1987-1991.

His main research area have been Biological Control and Integrated Pest Management. He has published two Books and a Monograph. He has several Patents and worked on 54 research projects in Canada, Uganda, Turkey, Rumania, Bulgaria, Indonesia, Sri Lanka and Sultanate of Oman.

**RECIPIENT OF  
ZOOLOGIST OF THE YEAR AWARD 2014\***



**Dr. Shamim Akhter,**  
*Associate Professor,*  
*Department of Zoology Arid Agriculture University, Rawalpindi*

Dr. Shamim Akhter joined Department of Zoology, PMAS-Arid Agriculture University in 1998, where she currently is Associate Professor of Zoology. Dr. Akhter received her M.Sc and M.Phil degrees from the University of Agriculture, Faisalabad and her Ph.D. from PMAS-AAUR in 2006. She did post doctorate from University of London, UK and international training in Egyptian International Centre for Agriculture and The Royal Veterinary College, London.

Dr. Akhter has contributed considerably both in teaching and research. Her research is mainly in the field of Animal Biotechnology, specifically the Gamete Preservation. Her expertise in the field of buffalo reproduction has significantly contributed to understand the underlying cause of poor conception rates in this species. Dr. Akhter has also made significant contributions in establishing Animal Physiology Laboratory at her campus. She has served as a Principal Investigator or a Co-Investigator in about ten research projects and secured handsome funding for research. She has published over 50 original peer reviewed articles in top journals in this field. Her articles have been cited numerous times by other investigators working in this area. In recognition of research publications, she has been awarded the

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\*Other nominee of this award were Prof. Dr. A.G. Arijo, Prof. Dr. Nusrat Jehan, Prof. Dr. Aleem A. Khan, Prof. Dr. Iftikhar Hussain, Dr. M. Ather Rafi, Dr. M. Hamid Bashir, Prof. Dr. Nasreen Memon and Dr. Muhammad Sarwar.

Research Productivity Award by Pakistan Council for Science and Technology in 2011 and 2012. Her excellent research has played a pivotal role to enhance the economics of buffalo production which is ultimately improving the life of livestock holders.

Being major supervisor she has trained well over 50 graduate students. Dr. Akhter's lab extends opportunities for advanced training in assisted reproductive techniques and also provides opportunity to graduates conducting bench work and learning the skills to publish articles in journals in the field of reproduction. She has delivered over 50 scientific presentations at various national and international meetings. She is the regional editor of International journal "Theriogenology Insight" and reviewer of journals of international repute.

Dr Akhter is an extraordinary and outstanding scientist of the highest caliber working in her field, distinguishing herself with remarkable research, and teaching skills. Her extraordinary achievements, remarkable skills and her outstanding career goals all warrant this award.

**RECIPIENT OF  
PROF. A.R. SHAKOORI GOLD MEDAL 2014\***



**Dr. Naveed Ahmed Khan**

*The Feerasta Family Chair & Professor, Department of Biological and  
Biomedical Sciences, Aga Khan University, Karachi.*

Prof. Dr. Naveed Ahmed Khan is a renowned microbiologist having contributed immensely in the field of Biological Sciences. After graduation in 1993 from the Bahauddin Zakariya University, Multan. Dr. Khan moved to the UK and completed his M.Sc. from the University of London and Ph.D. from the University of Hull. He has several years of research experience at the Tufts University School of Medicine, Boston, and at the Johns Hopkins University School of Medicine, Baltimore. In 2002, he moved back to the UK and joined the University of London as a Lecturer (Assistant Professor). For the next several years, he worked enthusiastically to pursue research and teaching, and became a Senior Lecturer in Microbiology (Associate Professor) in 2007. In 2008, he joined the University of Nottingham as an Associate Professor of Molecular Microbiology.

Dr. Khan returned to Pakistan in 2010 and was appointed as Professor and Chair, Department of Biological and Biomedical Sciences, the Aga Khan University. In this capacity, he has been actively involved in the planning, developing and implementation of academic and scholarly activities of the Department.

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\*Other applicants for this award were Dr. Riffat Sultana, Dr. Mukhtar A. Mahar, Dr. M. Fiaz Qamar, Dr. Sajid Nadeem, Dr. Syed Murtaza H. Andrabi, Dr. M. Hamid Bashir.



Dr. Naveed Khan's major interest in research and expertise centers around infectious diseases and antimicrobials. He has a remarkable publication record with over 200 peer-reviewed publications having cumulative impact factor >300. He has published 5 books by international publishers, 4 book chapters, has secured host of research grants [(>\$2 million obtained, also a number of proposals are under review by NIH (USA), HEC (Pakistan) and PSF (Pakistan)) and has supervised over 38 graduate students. He is an active reviewer for 70 journals, reviews grant proposals for 10 agencies and editor of prestigious journals including journal of Nature Publishing Group.

Dr. Naveed Khan has been the driving force behind developing and fostering cutting-edge, innovative research programs. He has developed innovative models of the blood-brain barrier to study pathogenesis of the central nervous system infections with an eye to identify potential therapeutic targets. One aspect of his work on cockroaches has identified that they are source of antimicrobials.

This work was presented at the Society of General Microbiology Conference in Nottingham and attracted worldwide media attention. Google search of "Naveed Khan and Cockroaches" yields more than 50,000 web pages on this topic. Several documentaries were made and shown on leading news channels, websites and peer reviewed journals on this topic. In addition, he is using innovative models of the blood-brain barrier to study pathogenesis of CNS infections. Dr. Naveed Khan has been interviewed on worldwide media channels. He has delivered over 126 presentations at national/international conferences; 82 invited talks and over 15 years of clear classroom communication.

**RECIPIENT OF  
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2014\***



**Dr. Mukhtiar Ahmed Mahar**

*Associate Professor,  
Department of Freshwater Biology & Fisheries, University of Sindh,  
Jamshoro.*

Dr. Mukhtiar Ahmed Mahar was born on 30<sup>th</sup> November 1973 in District Shikarpur Sindh and got the initial education in home district. Dr. Mahar received M.Sc. (1<sup>st</sup> class) degree in Freshwater Biology and Fisheries from University of Sindh, Jamshoro during 1995-1996. Dr. Mahar was awarded Vice-Chancellor's Medal for securing the 1<sup>st</sup> Class first position.

In 1998 he was appointed as Research Associate in the Department of Freshwater Biology and Fisheries, University of Sindh later on become the Lecturer during 2001 in the University of Sindh. Dr. Mahar obtained Ph.D. degree in 2004. Dr. Mukhtiar was appointed Assistant Professor in 2006. he also worked as Fisheries Specialist in Sindh Coastal Community Development Project for one year (2010). Later on he assumed the charge of the post of "Project Director Fisheries in Livestock and Fisheries Department, Government of Sindh until 2013. Presently he is working as Associate Professor in the Department of Freshwater Biology and Fisheries, University of Sindh. Jamshoro.

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\*Other applicant for this award were Dr. M. Fiaz Qamar

Dr. Mahar has published 38 research articles in internationally reputed journals in the field of Fisheries and Limnology. He has supervised a number of M.Phil and Ph.D. research scholars. Dr. Mahar is Life-Fellow of Zoological society of Pakistan. He has got the trainings in the field of Limnology and Fish Culture Development and attended seminars, symposia, workshops and conferences at national and international level.

**RECIPIENT OF  
PROF. IMTIAZ AHMAD GOLD MEDAL 2014\***



**Dr. Shafqat Saeed**

*Director*

*Bahadur Sub Campus, Bahauddin Zakariya University, Katchehry Road, Layyah*

Dr. Shafqat Saeed was born on November 1, 1969 in D.G. Khan. After his basic education D.G. Khan. He obtained B.Sc.(Hons) Agri. Entomology, in 1991 from Barani College Rawalpindi, and M.Sc.(Hons) in Entomology from University of Agriculture Faisalabad in 1994. He worked as Team Leader for two years in world Bank funded Research Project “House Hold Energy Strategy Study” (HESS). He was appointed Assistant Research Officer (ARO) at Entomological Research Sub-Station, Multan under Ayub Agriculture Research Institute Faisalabad from 1994 to 2004. He worked on Integrated Pest Management of wheat, cotton, vegetables and mango insect pests. He also worked on registration and label expansion of insecticides trials against insect pests of cotton. In 1999 he got fellowship in Research Project “Mass culture of pollinators for pollination of crops in Korea” and proceeded to Kyungpook National University, South Korea for his Ph.D. degree, under the supervision Prof. Dr. Yong Jung Kwon. He got experience of commercial rearing of *Osmia* bees for apple pollination, bumble bees for tomato pollination and honey bees as pollen collector and general pollinator for crop pollination. He completed his PhD degree and later continued for Post Doctorate fellowship for 10 months at Industrial Entomological Research Institute, Yechon, Korea. In 2004 he was selected as Assistant Professor in Department of Entomology, at College of Agriculture, Bahauddin Zakariya

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\*Other applicant for this award were Dr. Muhammad Razzaq, Dr. Muhammad Hamid Bashir.

University, Multan. There he started working on mango orchard management, especially on insect pests of mango, mango sudden death disease, its causal organisms *i.e.* bark beetles and their management. He discovered different Non Apis bees, syrphid flies, blow flies and honey bees as effective pollinators for different crops and fruit trees for enhancement of yield. His novel studies for identification of cotton mealy bug as a pest and its management has been appreciated and cited in the world. Resistance and cross resistance studies has also been carried out on cotton white flies and published in international journals. Basic studies for Red Cotton Bug identification, biology and management have been published in national and international journals. He trained thousands of Mango growers and cotton farmers through farmer field school approach, seminars and training workshops throughout Pakistan. He has been working as International and National Consultant of USAID for training of mango farmers and worked as Research Coordinator of Pakistan of Global pollination Project of FAO. He was working as Coordinator of Australian funded ACIAR project on Orchard Management of Mango pests especially on midges and bark beetles. He got research Productivity award of PCST for the years 2010, 2011 and 2012. He is an HEC approved supervisor for PhD students and produced four PhD students and eight PhD students are under his supervision. Fourteen students got M.Sc.(hons) degrees under his supervision. He successful completed 06 International and 07 National research projects one International and four National research projects are in progress.

Dr. Saeed participated in many national and international conferences and published 87 abstracts in proceedings. Whereas 72 original research papers have been published in peer reviewed journals. He has 310 citations with score of 09 h index. He wrote a chapter on Insect Pests in the Encyclopedia of “The Mango” being published by Royal Court of Sultanate of Oman. He organized a National Conference on theme of Food Security in Arid Agriculture and an international Seminar on Mango Sudden Death Disease Management in 2013. He has 22 years of research and teaching experience. Now he as working as Tenured Assistant Professor of Entomology and has additional charge of Principal, College of Agriculture, Layyah and Director BZU Bahadur Campus Layyah.

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

**1. Muzaffar Ahmad Gold Medal 2014**

Fifteenth Muzaffar Ahmad Gold Medal 2014 was received by Ms. Hina Manzoor for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.



**Hina Manzoor**

**2. Ahmed Mohiuddin Memorial Gold Medal 2014**

Tenth Ahmed Mohiuddin Memorial Gold Medal 2014 was given to Ms. Ms. Bakhtawar Soomro, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.



**Bakhtawar Soomro**

**3. Afsar Mian Gold Medal 2014**

Fifth Afsar Mian Gold Medal 2014 was given to Ms. Salaha Urooj who obtained first position in the M.Sc. Biology/Zoology examination of the Arid Agriculture University, Rawalpindi.



**Salaha Urooj**

**4. Muhammad Afzal Hussain Memorial Gold 2014**

Fourteenth Muhammad Afzal Hussain Memorial Gold 2014 was given to Ms. Munnazza Khatoon for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.

**5. Mujib Memorial Gold Medal 2014**

Eighteenth Mujib Memorial Gold Medal 2015 was given to Ms. Munnazza Khatoon, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

**6. Prof. Dr. S.N.H. Naqvi Gold Medal 2014**

Ninth Prof. Dr. S.N.H. Naqvi Gold Medal 2014 was given to Dr. Samina Arif for her Ph.D. degree in Zoology specializing in the field of Toxicology from University of Karachi, Karachi.

**7. Prof. Dr. S.S. Akbar Memorial Gold Medal 2014**

First Prof. Dr. S.S. Akbar Memorial Gold Medal 2014 was given to Mr. Sarfaraz Ali Sathio, who obtained first position in the M.Sc. Zoology examination with specialization in Entomology of the University of Sindh, Jamshoro.

**8. M.A.H. Qadri Memorial Gold Medal 2014**

Eighth Dr. M.A.H. Qadri Memorial Gold Medal 2014 was given to Dr. Wali Khan for his Ph.D. degree in Zoology specializing in the field of Parasitology from University of Karachi, Karachi.



**Dr. Wali Khan**



**ISOLATION OF A GROWTH HORMONE VARIANT FROM  
PITUITARY OF WATER BUFFALO *BUBALUS BUBALIS*, IDENTICAL  
TO GROTH HORMONE OF SEI WHALE (*BALAENOPTERA BOREALIS*)**

AMTUL JAMIL SAMI<sup>1\*</sup> AND A.R. SHAKOORI<sup>2</sup>

<sup>1</sup>*Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore*

<sup>2</sup>*School of Biological Sciences, University of the Punjab, New Campus, Lahore*

**Abstract.-** A bubaline growth hormone (GH) variant was isolated from pituitary extract of *Bubalis bubalus*. The variant was identified by immunoblotting and mass spectroscopy after SDS-PAGE. Total protein in fresh pituitaries from water buffalo (collected from the local Slaughterhouse, Baker Mandi, Lahore) were homogenized in buffer pH 9.5. The total soluble proteins were fractionated on SDS-PAGE. A 20K protein band was identified as GH like protein by immunoblotting. The protein band was excised and analyzed by mass spectroscopy after tryptic digestion. The results showed that GH like band had two sequences for the position 96-100 1, RVFTNS identical to bubaline growth hormone and 2, KAYANE amino acid residues identical to *Balaenoptera borealis* Sei Whale GH for the same position (Accession No. ACP33092). The identified sequence is submitted to EMBL under as accession number, P86227 for GH variant 1 (Somatotropin 2) from *Bubalus bubalis*. Identical results were obtained with three different sets of experiments for bubaline pituitary growth hormone proteomics.

**Key words:** Growth hormone, growth hormone variant, *Bubalus bubalis*, *Balaenoptera borealis*, Sei whale.

## INTRODUCTION

Growth hormone (GH) is a member of cytokine growth factor super family among proteins with a unique 3 dimensional structure, comprising four helices bundle with unparalleled connectivity (Abdel-Meguid *et al.*, 1987; de Vos *et al.*, 1992; Kossiakoff and de Vos, 1999). GH is produced by the pituitary and placenta in mammals. It is related to prolactin and somatolactin, which are other members of GH-prolactin family. GH regulates growth after binding with two receptor molecules on the target tissue and activate a number of anabolic pathways involving tyrosine kinase Jak2 (Herrington and Carter-Su, 2001). Expression of more than one GH gene in ruminants was demonstrated in goat, sheep, cow, hippopotomus and giraffe (Maniou *et al.*, 2004; Valinsky *et al.*,

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1990; Wallis *et al.*, 2001; Wallis and Wallis, 2001; Sami, 2010; Sami *et al.*, 2011). In this connection, a bGH variant with a mutation at position 126 for Val/Leu was studied in detail. This variant has a relationship with the higher levels of GH in the farm animals for higher milk yield. This is identified as a physiological marker for higher milk yield (Løvendahl and Sejrsen, 1993; Løvendahl *et al.*, 1991, Lucy *et al.*, 1993; Vukasinovic *et al.*, 1999).

A cow GH gene is a single copy gene which spans 1.8 KB with 5 exons and four introns (Valinsky *et al.*, 1990; Woychick *et al.* (1982). Woychick *et al.* (1982) identified a number of possibilities for the expression of bovine GH variants due to mRNA splicing, as in the case of 20k hGH (with a deletion of 15 amino acid residues due to mRNA splicing) and 17.5k hGH in case of bovine GH genes. There are possibilities, but no variant is detected yet (Valinsky, 1990). Recently, with the use of computational tools, it was known that there is a potential for the expression of 20k hGH like bGH protein (as there is consensus for mRNA splicing pattern in the gene), the expressed product with deletion of amino acid residues 33-46 has altered solubility (Sami, 2010). Structure and function of different motifs of GH were studied in detail by Chen *et al.* (1995), Sami (2007) and Wallis and Wallis (2001).

Region 96-100 has not been identified for any functional activity. Holder and Beattie (1998) had studied the amino acid residues 91-102 for a startling discovery that the antigenic molecules generated against different fragments (including 96-100 for bGH/oGH) of the region potentiate the growth promoting activity of GH, though the level of potentiation is different. GH has a strongly conserved sequence of nucleic acid and amino acid level (Maniou *et al.*, 2004). The eruption of a new order Cetariodactyla has provided some information about an irregular molecular evolution of pituitary GH. Graur and Higgins (1994) reported molecular evidence for the inclusion of the Cetaceans within the order Artiodactyla. Evolutionary studies on the origin of whales and it has now been proven that the earlier artiodactyls are the ancestors of sea whale (Gingerich *et al.*, 2001). For the origin of whales from early artiodactyls, the subcontinent in the Indo-Pakistan were reported (Gingerich *et al.*, 2001). Thewissen *et al.* (2007) reported that whales originated from aquatic artiodactyls in the Eocene epoch of India. In another report Thewissen and Hussain (1993) discussed the origin of underwater hearing in whales. Graur and Higgins (2004) reported links between sea mammals and ruminants on the basis of paleontological evidence. We have isolated a GH variant from the crude extract of water buffalo with a motif uniform of Sei Whale (*Balaenoptera borealis*). This variant has not been reported

before and may require some evidence to create a link between sea mammals and ruminants.

## METHODS

Samples were collected from freshly slaughtered water buffalo from Bakar Mandi Lahore, Pakistan. The protein extract was prepared by homogenizing the sample in 0.05 M Na carbonate-bicarbonate buffer, pH 9.5. A weighed amount of pituitaries (50 g) was washed with distilled water and homogenized in 0.05M sodium carbonate/sodium bicarbonate buffer (pH 9.5). The crude protein was precipitated with 90% saturation of ammonium sulfate and centrifuged at 5000xg for 10 minutes. The pellet obtained was dissolved in 50 ml of 0.05M bicarbonate buffer pH 9.5.

The protein solution was dialyzed against the same buffer. The clear supernatant obtained after centrifugation was used as a source of protein. The sample was purified as described previously (Sami, 2006), by using a sequential purification procedure including ion exchange chromatography and preparative SDS-PAGE. The protein extract and purified protein were subjected to SDS-PAGE in duplicates, by loading 20 µg of protein on 10% polyacrylamide electrophoresis gel. After electrophoretic run, one half of the gel was stained for proteins with Coomassie blue dye (1% solution in 20 ml methanol and 10 ml acetic acid). After destaining blue bands were visible against a clear background. Second half of the gel was used for transferring protein onto a nylon membrane over night for immune blotting. The membrane was washed three times with PBST (containing 1.0% skimmed milk for blocking nonspecific binding of proteins) buffer for 10 minutes each. Then membrane was incubated with a primary antibody solution (100 micro liter diluted in 10 ml PBS) that was antigrowth hormone antibody for overnight with constant shaking. After washing three times with PBS, secondary antibody antirabbit IgG (conjugate to horsereddish peroxidase) was added and incubated for one hour.

The membrane was washed with PBS. It was dried on filter papers and was transferred to a petri plate containing Guaiacol (Sigma). The membrane was removed and dried on filter paper and then transferred to another Petri plate containing turnip peroxidase enzyme. The membrane was thoroughly soaked in enzyme solution, and 3-4 drops of hydrogen peroxide were added. Growth hormone band appeared on membrane in light brown color against a pink background. The immunoblot was used to locate the position of growth hormone like a band on SDS- gel. GH band was identified on SDS gel, and a sharp razor

was used to sliced the GH band. The band was stored in 1ml double distilled deionized water and sent for protein identification by spectroscopic analysis.

## RESULTS AND DISCUSSION

Pituitary samples were collected from freshly slaughtered water buffalo, *Bubalus bubalis* from Bakar Mandi Lahore, Pakistan. The protein extract was prepared by homogenizing the sample in 0.05 M  $\text{Na}_2\text{CO}_3$ - $\text{NaHCO}_3$  buffer, pH 9.5. Extract of freshly collected pituitaries was prepared as described in methods. The pH was adjusted to 7.0 and was concentrated with 30% ammonium sulfate. After dialysis and centrifugation the clear supernatant was subjected to SDS-PAGE and immunodetection. The sample was purified as described previously (Sami, 2006). Crude and purified samples were subjected to SDS-PAGE. The purified fraction single band was visible (Fig. 1, Lane 2 and 3) at the position equivalent to position 22 Kd (closer to the molecular weight of bubaline GH (Fig. 1, lane 3). The GH bands were identified by immunoblotting using anti GH antibodies (Fig. 1 lane 4 and 5). After identification by immunoblotting the protein bands were excised from the gel and sent for protein identification by spectroscopic analysis to the St. Andrews University, Spectroscopy Lab, Scotland, UK. The identified fragments of GH variant are shown in Figure 2.

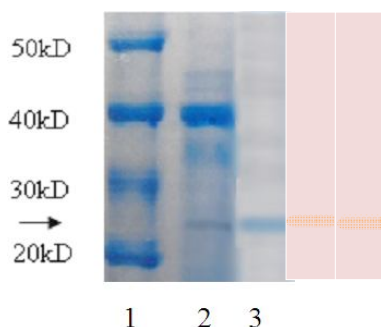


Fig. 1. SDS – PAGE and Immunoblotting of crude and purified pituitary bubaline GH (arrow shows the position of GH and GH variant). Lane 1, Molecular weight marker; Lane 2, Sample after ammonium sulfate precipitation; Lane 3, Purified GH protein after ion exchange (described in Section 2); Lane 4, Immunoblot of sample after ammonium sulfate precipitation; Lane 5, Immunoblot of purified GH protein after ion exchange.

The protein band was analyzed by spectroscopic methods as GH. The data analysis showed the identification of two different amino acid sequences as in

position 96-100, one is (i) RVFTNS and the other is (ii) KAYANE (Fig. 2), for bubaline GH. RVFTNS was identical to the GH sequence that has been reported by Verma *et al.* (1999) while the KAYANE sequence showed identity to Sei whale GH in the region of 96-100 amino acid residues (Fig. 2). The purified protein did not show the sequence identical to Sei whale GH. There was 83 % homology among Bubaline and Sei whale GH, while the newly isolated variant showed greater homology than Sei whale. The experiment was repeated 3 times with different samples at different times after an analysis KAYANE peptide fragment appeared in all samples. A close look at the three dimensional structure of the molecule it was revealed that the region (96-100) forms a part of helix 2 and a part of the loop between helix 2 and helix 3 (Fig. 3) and the region is exposed. A comparison of both the fragments shows the addition of highly charged amino acid glutamic acid instead of serine which lead to lowering of the isoelectric point of a protein. This change provides an acidic environment to the molecule and perhaps introduce new hydrogen bonds within the molecule and with the receptors, the motif 96-100, is found near the binding sites with the receptor 1 of GH molecule (GH molecule binds with two receptors for its biological activity). Other variations including replacement of threonine with Isoleucine, at position 71 (as shown in Fig. 2) may not have any considerable effect on the global chemistry of the molecule. Region 96-100 in the bGH and oGH has not been identified for any functional activity. Chen *et al.* (1995) had identified amino acid residues in the third alpha-helix of GH involved in growth promoting activity. Holder and Beattie (1998) have reported that the generation of antibody against the motif could enhance the biological action of GH. Perhaps this could be related to the masking of the region (96-100) which is exposed in the molecular model of the molecule (Fig 3, shown in blue). This could encounter the steric hindrance for binding of GH molecule with its receptor molecules, by masking the region. Another change was observed in helix 4 is Isoleucine instead of leucine, while this change does not effect on the global chemistry of the molecule. It is pertinent to mention that the exogenous supply can increase the linear growth rate in farm animals (Bauman, 1999; Bauman and Vernon, 1993; Lucy *et al.*, 1993).

Expression of a bubaline GH variant with a motif KAYANE for the position 96-100 could be a result of gene duplication and the presence of another allele of GH gene. Earlier duplication of GH gene in ruminants e.g. cow, goat, giraffe and hippo has been reported (Maniou *et al.*, 2004; Wallis *et al.*, 1998). Though the gene duplication has been reported in the giraffe and hippo, but the duplicate gene could not be isolated as reported by Maniou *et al.* (2004). Though, several attempts identified the duplicate gene in bubaline mRNA but were not

successful (data not shown). Genomic studies could be helpful in finding the origin of the second gene, as in case of cow genome a number of genes coding for GH has been identified (Maniou *et al.*, 2004). It may be noted that amino acid sequence GH from *B. Borealis* was reported by identifying amino acid after protein purification. There is no DNA sequence available for the protein. Our results repeated three times with different samples support the sequence available for *B. borealis* GH protein. We are trying to isolate the gene for the protein sequence, so far there is not much success, possibly due to gene duplication with the gene with minor changes

Buffalo	AFPAMSLSGLFANAVLRAQHLHQLAADTFKEFERTYIPEGQRYSIQNTQV
Sei	-FPAMPLSSLFANAVLRAQHLHELAADTYKEFERAYIPEGQRYFLQNAQS
varinat	AFPAMSLSGLFANAVLRAQHLHQLAADTFKEFERTYIPEGQRYSIQNTQV
	****.*.*.*****:*****:*****:*****:***.*
Buffalo	AFCFSETIPAPTGKNEAQQKSDLELLRISLLLIQSWLGPLQFLS <u>RVFTNS</u>
Sei	TGCFSEVIPTPANKDEAQQRSDVELLRFSLLLIQSWLGPVQFLE <u>KAYANE</u>
varinat	AFCFSETIPAPTGKNEAQQKSDLELLRISLLLIQSWLGPLQFLS <u>SKAYANE</u>
	:****.*.*.*.*:*****:*****:*****:*****:*****:***.:*.*.*
Buffalo	LVFGTSDRVYEKLDLEEGILALMRELEDVTPRAGQILKQTYDKFDTNMR
Sei	LVFGTSDRVYEKLDLEEGIQALMRELEDGSPRAGQILKQTYDKFDTNMR
varinat	LVFGTSDRVYEKLDLEEGILALMRELGDVTPRAGQILKQTYDKFDTNMR
	***** * :*****
Buffalo	SDDALLKNYGLLSCFRKDLHK <u>T</u> ETYLVRVMKCRRFGEASCAF
Sei	SDDALLKNYGLLSCFKDLHK <u>A</u> ETYLVRVMKCRRFVESSCAF
varinat	SDDALLKNYGLLSCFRKDLHK <u>T</u> ETYLVRVMKCRRFGEASCAF
	*****:***** ***** *:****

Fig. 2. Alignment of bubaline GH peptide with Sei Whale GH and newly identified bubaline GH variant. Variations in the amino acid sequence for bubaline GH and bubaline GH variant are under lined and in italics.

The evolution of GH has been reviewed in detail (Maniou *et al.*, 2004; Wallis *et al.*, 1998, Wallis and Wallis, 2001) have reported a burst of rapid changes in the GH amino acid sequence leading to episodic evolution (within the newly erupted order Cetartiodactyla comprising Artiodactyla and Cetacea). The region 96-100 is reported to be highly conserved among all other cetartiodactyls with an exception of sei whale. In Giraffe, there is only one change in the region for position 99 where threonine is replaced by serine (Maniou *et al.*, 2004). Insertion of a part of the sequence from cetacean could lead to a notion that as artiodactyls in the region of the Indian subcontinent are ancestors of cetaceans and terrestrial artiodactyls (water buffalo) at some stage of evolution. This could

provide a clue, about the link between Artiodactyla and Cetacean, based on the paleontological findings that the origin of whale is linked to the early artiodactyls (Gingerich *et al.*, 2001; Nummela *et al.*, 2007). There is a possibility that the insertion of the sequence from the sei whale in the bubaline GH or vice versa could be an event during evolution. Conclusively, we found a growth hormone variant in *Bubalus bubalis* pituitary extract showing identity to the Sei whale GH, particularly in the region of 96-100. To quest the fate of bubaline GH variant genomic studies could be helpful.

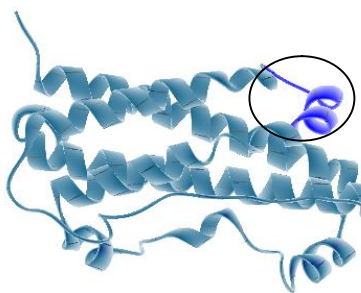


Fig. 3. Three dimensional structure of bubaline growth hormone, position of amino acid 96-100 is shown in dark (encircled area) forming a part of the loop between helix 2 and helix 3 and a part of helix 2

### ACKNOWLEDGEMENT

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## BIODIVERSITY AND FISHERIES POTENTIAL OF LAKE GAHOT, MATIARY, SINDH, PAKISTAN

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**Abstract.-** Lake Ghahot is located 25°56'04.70"N and 68°25'55.44"E on the global map. It covers the area of about 200 acres with an average depth about 25-32 ft. Water quality and biological samples were collected during 2013. Temperature of water was found between 15 to 28°C, visibility in water was observed 0.7 to 1.3 m, pH ranges were observed 8.4 to 8.9; alkalinity and hardness (CaCO<sub>3</sub>) were noted 198 to 245 mg/l and 217 to 320 mg/l respectively. Salinity, conductivity and TDS were noted 1.9 to 2.8 ppm, 2141 to 5250 µS/cm, and 1204 to 2573 mg/l respectively; dissolved oxygen ranges were recorded from 7 to 12.5 mg/l. Thirty two species of phytoplankton and algae belong to phylum Cyanophyta, Chlorophyta and Bacillariophyta were recorded. The filamentous attached floating genera of algae *Cladophora*, *Mougeotia*, *Oedogonium*, *Spirogyra* and *Hydrodictyon* were commonly found at shallow areas. Twenty species of zooplankton were observed, among them the *Diaphanosoma*, *Simocephalus*, *Mesocyclops*, *Cyclops*, and *Neodiaptomus* genera were found dominant during spring and summer seasons. Ten species of macrophytes were reported from the lake. Twenty six species of fish belonging to carps, catfishes, spiny eels, snakeheads and tilapia were reported. The total catch from all landing centers was 45.5 metric tons/annum. The fish production and natural productivity of lake Ghahot was found adequate in relation to the limnological parameters.

**Keywords:** Fisheries, limnology, Lake Ghahot.

### INTRODUCTION

Very few aquatic resources, in the form of private and public waters are present in district Matiary, Sindh, Pakistan. The Indus River forms a large depression near village Bhanoth called Rechal Porath. This large depression is main source of fish seed production. The lake Ghahot is present in Matiary district on location (longitude 25°56'04.70"N and 68°25'55.44"E and altitude) of Simroon bund left side of river Indus near village Allah Rakhyo Mallah and Otaq Pir Fazal Haq, Saeedabad Matiary. The lake covers the area of about 200 acres with an average depth of about 12-18 ft. The lake is connected with Dhandh Purano through the Gharo wah just at northern side and Indus river flows at western side. The water source of the lake is from Ghahot canal which is a crossing at southern region. The lake is moderately nursery of natural fish seed

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and fingerlings in period of the after monsoon season due to direct connection with river Indus. The formation of lake Gahot is not much old, this depression was actually the part of river Indus; with the passage of time the flow of river Indus diverted time to time in result it left a number of small depressions to its commending area. The lake Gahot is not investigated previously; the present limno-biological study is the first of its type that is being revealed the status of aquatic fauna, flora and fisheries of the lake. This baseline studies will be beneficial for further development for fisheries sector in the region.

### **MATERIALS AND METHODS**

The survey program for collection of water samples, plankton, macrophytes and fish was scheduled in 2013. Four sampling sites were selected, with the help of GPS device for collection of samples and on-spot investigation and physical observation in the lake Gahot. The sketch of the lake was downloaded from Google earth. Temperature of air and water was noted with mercury thermometer. Transparency of water, rate of suspended solids and light penetration in water was measured with the help of a metal black and white disc that was lowered in water; result was recorded at the point of appearance and disappearance. The algae and plankton samples were collected with the help of plankton net (No.25, 55 $\mu$ ), preserved in 5% formaline. Macrophytes and fish samples were collected with the help of nets and plant gripper, fish species were preserved in sterilized plastic bottles in ice box in the field while plants were preserved in plastic bags. The further analysis and studies of samples were carried out in the laboratory. The water quality parameters were examined from collected samples with the help of Orion 5 Star pH-ISE-Cond-DO Benchtop Thermo Scientific digital meter on spot. Alkalinity, hardness were detected through the standard titration methods of HECH Kit.

### **RESULTS AND DISCUSSION**

Physico-chemical parameters of water and biological samples of lake Gahot were collected and studied to find out the eco-biological status. Temperature of water was found between 15 to 28°C, visibility in water was observed 0.7 to 1.3 m, pH ranges were observed 8.4 to 8.9; alkalinity and hardness (CaCO<sub>3</sub>) were noted 198 to 245 mg/l and 217 to 320 mg/l, respectively. Salinity, conductivity and TDS were noted 1.9 to 2.8 ppm, 2141 to 5250  $\mu$ S/cm, and 1204 to 2573 mg/l, respectively. Dissolved oxygen 9 to 12.5mg/l was recorded (Table I).

TABLE I.- WATER QUALITY PARAMETERS OF PUBLIC WATERS MATIARY 2013.

Parameters	Winter (Jan- Feb)	Spring (Mar-Apr)	Summer (May- Aug)	Autumn (Sep-Dec)
Temperature (°C)	15	22	28	16
Transparency (m)	0.7	0.7	1.3	0.9
pH	8.9	8.4	8.6	8.8
Alkalinity (mg/l)	212	198	204	245
Hardness (mg/l)	320	265	217	275
Salinity (ppm)	2.8	1.3	1.9	1.95
Conductivity (µS/cm)	5250	2456	2141	2188
TDS (mg/l)	2573	1204	2113	2146
Dissolved oxygen (mg/l)	12.2	9.5	7.5	10

Results of water quality parameters in lake Gahot were found quite suitable for existence of freshwater fish of any type due to enough water entrance in the lake directly during flooded season, thus support the freshwater biota. Water quality parameters of other lakes of Sindh were studied by different workers and the present findings are also supported by various studies conducted by Baqai *et al.* (1974a), Baqai *et al.* (1974b) and Mahar *et al.* (2000a) on different water bodies connected with river Indus. Surrounding anthropogenic communities are using the lake water for domestic purposes. The study included the annual cycle of physico- chemical variables and the population of zooplankton, its qualitative and quantitative composition. In another studies of Mahar *et al.* (2007), on the ecobiological conditions of Chotiari reservoir concluded that the water quality is suitable for carp fish culture. The same situation was observed in lake Gahot's present studies, the ratio of a number of carp trash fish and nursery sized fish (2-3 inches) were recorded abundantly due to suitability of water quality and natural productivity. Thirty two species of phytoplankton and algae related to phyla Cyanophyta, Chlorophyta and Bacillariophyta were recorded from collected samples from three sampling station, among them chlorophycean species were commonly recorded in lake Gahot, while very few species of Cyanophyta were observed. The filamentous attached floating genera *Cladophora*, *Mougeotia*, *Oedogonium* and *Spirogyra* were commonly found in all sampling stations. Only four species of diatoms *Gyrosigma*, *Nitzschia*, *Navicula* and *Pinneularia* were recorded in samples of Dhandh Gahot as the indication of freshwater (Table II).

The present studies of lake Gahot are confirmed with the findings of Nazneen (1974) in Keenjhar lake it was found that maximum bloom of phytoplankton occurred during summer to high growth of Myxophyceae, while in Bacillariophyceae growth was greater than Chlorophyceae, a decrease in the abundance of total phytoplankton observed in May and in November was

TABLE II.- PHYTOPLANKTON RECORDED FROM LAKE GAHOT IN MATIARY 2013.

Genera	Winter (Jan- Feb)	Spring (Mar-Apr)	Summer (May- Aug)	Autumn (Sep-Dec)
<i>Microcystis incerta</i>	+	+	+	+
<i>Microcystis flos-aqua</i>	+	+	+	+
<i>Merismopedia glauca</i>	+	+	+	+
<i>Merismopedia minima</i>	+	+	+	+
<i>Anabaenopsis arnoldii</i>	-	-	+	+
<i>Anabaena planktonica</i>	-	-	+	+
<i>Anabaena limnetica</i>	-	-	+	+
<i>Anabaena sphaerica</i>	-	-	-	+
<i>Calothrix marchina</i>	-	-	-	+
<i>Spirulina gigantea</i>	-	-	-	+
<i>Arthrospira massarii</i>	-	+	-	-
<i>Oocystis elliptica</i>	+	+	-	-
<i>Oocystis pusilla</i>	+	+	+	-
<i>Oocystis borgei</i>	+	+	+	-
<i>Cylindrocystis meneghini</i>	+	+	+	-
<i>Scenedesmus quadricauda</i>	-	+	++	-
<i>S. bijugatus</i>	-	-	+	+
<i>Pediastrum simplex</i>	-	-	++	+
<i>P. tetras</i>	+	-	+	+
<i>Tetraedron gigas f. minus</i>	+	-	+	+
<i>Cosmarium succisum</i>	-	-	++	+
<i>C. granatum</i>	-	-	++	+
<i>Cladophora</i>	++	+	+	+
<i>Mougeotia</i>	++	+	+	+
<i>Oedogonium</i>	++	+	+	+
<i>Spirogyra</i>	++	+	+	+
<i>Lepocinclis playfairiana</i>	-	+	++	+
<i>Navicula viridula</i>	+	+	+	-
<i>Amphora ovalis</i>	+	+	+	-
<i>Gyrosigma acuminatum</i>	+	+	+	-

attributed to the disappearance of *Microcystis aeruginosa*. The lake Gahot is rich with algal population and best nursery for fingerling's feeding. Twenty species of zooplankton were recorded from lake Gahot including major genera of *Diaphanosoma*, *Simocephalus*, *Mesocyclops*, *Cyclops*, and *Neodiaptomus*. The presence of these cladoceran and copepod species at abundant level in the lake environment is the clear indication of freshwater (Table III). It is natural phenomenon that as micro nutrients increase the phytoplankton increase, which followed by Zooplankton. Rich population of zooplankton was reported from lake Gahot. Same types of studies were carried out by different workers on the other water bodies of Sindh; Iqbal and Baqai (1975) recorded preliminary observations on seasonal abundances of cladoceran genera in Keenjhar lake and

concluded as transparent freshwater lake. The zooplankton population was occurred in August-September while the second peak of the cladoceran population was occurred in January and February; the high population of cladoceran may be correlated with temperature, breeding season and migration of fishes to deeper water during winter. Baloch (2000) reported 28 species of phytoplankton and 26 species of zooplankton from Indus river and revealed the suitability of freshwater in the river Indus.

TABLE III.- ZOOPLANKTON RECORDED FROM LAKE GAHOT IN MATIARY 2013.

Genera	Winter (Jan- Feb)	Spring (Mar-Apr)	Summer (May- Aug)	Autumn (Sep-Dec)
<i>Brachionus forficula</i>	++	+	+++	+
<i>Brachionus quadridentatus</i>	++	+	++	+
<i>Brachionus plicatilis</i>	+	+	+	+
<i>Filinia longiseta</i>	+	+	+	+
<i>Filinia terminalis</i>	+	+	+	-
<i>Keratella tropica</i>	+	-	+++	-
<i>Lecane luna</i>	-	-	+	-
<i>Lecane niothis</i>	-	-	+	++
<i>Lecan inopneta</i>	-	+	+	-
<i>Monostyla bulla</i>	++	+	+	-
<i>Testudinella mucronata</i>	-	+	+	+
<i>Phylodina erythrophthalma</i>	-	+	++	+
<i>Alona rectangula</i> Sars	-	+	++	+
<i>Bosminopsis deitersi</i>	+++	+	+++	+
<i>Bosmina longirostris</i>	+	+	+	-
<i>Ceriodaphnia reticulata</i>	+	+	+	-
<i>Ceriodaphnia cornuta</i>	-	+	+	-
<i>Ceriodaphnia setosa</i>	-	+	+++	++
<i>Diaphanosoma sarsi</i>	+	-	+	-
<i>Moina rectirostris</i>	-	-	++	-

Mahar *et al.* (2000) reported diversity and seasonal occurrence of planktonic rotifers from lake Manchar and concluded as the lake is eutrophic on the basis of rotifer species and water quality parameters. The seasonal variation and species composition of various zooplankton forms were studied by Mahar *et al.* (2007) and Mahar *et al.* (2008) and concluded that a number of zooplankton forms are indication the water quality status. The present findings are correlated with above findings. Ten species of macrophytes were recorded from lake Gahot. The presence, abundance, and dispersal of higher aquatic plants indicate the quality of water in relation to oligotrophic, mesotrophic and eutrophication (Mahar *et al.*, 2000). In the present studies the qualitative and quantitative analysis of plants indicated that the lake Gahot environment is at the stage of mesotrophic (Table IV).

TABLE IV.- HYDROPHYTES RECORDED FROM LAKE GAHOT IN MATIARY 2013.

Genera	Winter (Jan- Feb)	Spring (Mar-Apr)	Summer (May- Aug)	Autumn (Sep-Dec)
<i>Phragmites vallatoria</i>	+	+	+	+
<i>Typha domigensis</i>	+	+	+	+
<i>Ipomoea aquatica</i>	+	+	+	+
<i>Eichhornia crassipes</i>	+	+	+	+
<i>Nelumbo nucifera</i>	-	+	++	-
<i>Nymphia lotus</i>	-	+	+	-
<i>Hydrilla verticillata</i>	+	-	-	+
<i>N. japonica</i>	+	-	-	+
<i>Potamogeton pectinatus</i>	-	++	+	-
<i>P. crispus</i>	-	-	+	-

The occurrence of macrophytes in other lakes of Sindh which are connected with river Indus are reported by Leghari *et al.* (1999), Leghari *et al.* (1999) and Jahangir *et al.* (2013). Nazir and Younis (1979) reported the macrophytes from Punjab Pakistan. Twenty six species of fish consisting carps, catfishes, spiny eels, snake heads, tilapia and a number of trash fish were recorded (Table V). Qualitative and quantitative studies on freshwater fish belonging to different water bodies are reported by Mirza (1982) Jafri *et al.* (1999) and Mahar *et al.* (2000). Lake Gahot is semi perennial public water thus a number of boats are engaged for catching fish on daily basis. Lake Gahot has direct connection with river Indus therefore same fish fauna was observed. An average production of tilapia was observed from 2.3-2.82 mt it is interesting that the tilapia is only group of fish which was caught regularly, the second crop was of snakeheads were 1.1-3.26mt and the third was the cat fish 1.31-3.21 mt. Highest production of carp fish was observed in winter season 2.1-4.32 mt during 2013. The total fish production was ranged from 8.420- 15.92 mt with grand total of whole lake Gahot was found 45.4 mt in 2013 (Table VI). present results of fish production in lake Gahot are quite satisfactory due to direct connection with river Indus when compared with the results of other water bodies and lakes of Sindh carried out by Jafri *et al.* (2000) and Mahar *et al.* (2000). Matiary district is small in relation to its revenue area but rich in relation to fish and fisheries potential from other five public water including river Indus. The ecological conditions of lake Gahot are quite suitable for aquaculture management. The present fish catch of the lake is not enough to its total occupied area. It is therefore suggested that more stock of major carps should be released in to the lake for proper utilization of the natural productivity and area of the lake.

TABLE V.- FISH SPECIES RECORDED FROM LAKE GAHOT IN MATIARY 2013.

Genera	Winter (Jan- Feb)	Spring (Mar-Apr)	Summer (May- Aug)	Autumn (Sep-Dec)
<i>Gudusia chapra</i>	+++	+++	++	+
<i>Barbus sarana</i>	+++	++	+	+
<i>Catla catla</i>	+	-	-	+
<i>Cirrhinus mrigala</i>	+++	-	-	+
<i>Cirrhinus reba</i>	++	-	-	+
<i>Labeo rohita</i>	+++	-	-	+
<i>Labeo calbasu</i>	+++	-	-	+
<i>Labeo gonius</i>	+++	-	-	+
<i>Puntius sophore</i>	+++	-	-	+
<i>Puntius ticto</i>	+	+	-	
<i>Salmostoma bacaila</i>	+++	++	+	++
<i>Aorichthys aor</i>	++	+	+	++
<i>Bagarius bagarius</i>	+++	+	+	+
<i>Eutropiichthys vacha</i>	+++	+	+	++
<i>Mystus seenghala</i>	+++	++	+	++
<i>Mystus cavasius</i>	+++	++	+	+++
<i>Ompok bimaculatus</i>	+	+++	+	++
<i>Rita rita</i>	+++	+	+	++
<i>Wallago attu</i>	++	+++	+	++
<i>Notopterus notopterus</i>	+++	++	+	++
<i>Notopterus chitala</i>	+++	+++	+	++
<i>Channa marulius</i>	+++	++	+	+
<i>Channa striatus</i>	+++	++	+	+
<i>O. mossambicus</i>	+++	+++	+++	+++
<i>Tilapia zilli</i>	+++	+++	+++	+++
<i>Mastacembelus armatus</i>	++	+	+++	+

Signs and symbols:

+, Present; ++, Common; +++, Abundant; -, Absent

TABLE VI.- FISH PRODUCTION (M. TONS) OF LAKE GHAHOT IN MATIARY 2013.

Fish group	Winter (Jan- Feb)	Spring (Mar-Apr)	Summer (May- Aug)	Autumn (Sep-Dec)	Total
Carp fish	4.32	2.1	2.13	3.29	11.84
Cat fish	3.21	1.31	1.61	1.45	7.58
Snakehead fish	3.26	1.1	1.94	2.98	9.28
Tilapia fish	2.82	2.51	2.57	2.3	10.2
Trash fish	2.31	1.4	1.26	1.53	6.5
Grant total	15.92	8.42	9.51	11.55	45.4

Source: Livestock &amp; Fisheries Department, Government of Sindh.



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## EVALUATION OF GROUND WATER QUALITY OF TEHSIL BARNALA, DISTRICT BHIMBER, AJ&K, PAKISTAN

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**Abstract.-** Present study was designed to determine the ground water quality of Tehsil Barnala. A total of 7 water samples were collected from Tehsil Barnala District Bhimber to analyze for various physicochemical and biological parameters i.e. namely temperature, pH, turbidity, color, odor, taste, electric conductivity (EC), total dissolved solids (TDS), total hardness (Calcium + Magnesium), chloride, arsenic, nitrite and *Escherichia coli*. Results revealed that ground water of Tehsil Barnala is grossly contaminated with pathogenic microorganisms like *E. coli*. Besides it, values of some physicochemical water quality determining parameters were also beyond the limits suggested by World Health Organization (WHO) i.e. Chloride ion concentration was below the prescribed limits. It has been proven that consumption of unsafe drinking water is one of the major causes of prevalence of water born diseases like diarrhoea, typhoid fever and malaria in the study area.

**Key words:** Microbial contamination, ground water pollution.

## INTRODUCTION

Water, the most abundant compound on the planet Earth is essential for all living organisms. Its uses in all living things cover a huge variety of everyday functions, which are important to the continuity of the organism. All organisms on Earth somehow depend on water, and if the water they consume is adulterated, it may result in fatal changes in consuming organisms' lives. There are two main sources of water i.e. surface water sources (such as rivers stream and lakes) and the ground water (includes well water and bore hole water) (McMurry and Fay, 2004).

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During recent decades deficiency in surface water reservoirs occurred because of excessive agricultural use and population growth. This situation changed the exploitation policy of water resources as a result reliance on ground water increased. The revolution of ground water development for irrigation started in Italy, Spain, United States and Mexico. The second wave of ground water development for irrigation started in South Asia, parts of north China plains, parts of middle East and in North Africa during 1970 and it still continues today. The ground water experts forecast a third wave of revolution in many regions of Africa, Srilanka and Veitnam. This boom was named Silent Revolution and is based on individual decision by farmers and did not include any federal planning (Shah *et al.*, 2007). The silent revolution resulted an increase in ground water withdrawal across the globe, due to which a stress produced on ground water system, when rate of withdrawal crossed the rate of mean ground water restoration (Llamas and Martinez-Cortina, 2009; Llamas and Martinez-Santos, 2005).

The decline of water table results in increase of carbon emission and consequently raises pollution. There is approximately 4.8% boost in carbon emission for each meter drop in ground water level. Carbon emission cause global warming due to which variation in pattern of precipitation and evaporation takes place, that result severity of floods and rise in sea level. Rise in sea level cause introduction of saline water into fresh ground water and ultimately ground water quality is badly affected (Mall *et al.*, 2006). About 800 million people in developing countries did not have availability of safe drinking water (WHO, 1988). World Water Council stated in 1999 that at least 95% of human beings must have access to clean drinking water by 2025. It is reported that about 35% of the World's population is suffering from serious water stress conditions (Alcamo *et al.*, 2000).

A year wise estimate of global mortality rate per year from water born diseases is as follows, 2.3 million mortality rate (WHO, 1999), more than 5 million global death rate (WHO, 1996), more than 3 million deaths occur globally due to water related diseases (Water Dome Report, 2002; WHO and UNICEF, 2004).

The ground water pollution has reached at alarming point in Pakistan. The situation demands activation of water purifying measures at emergency basis. The present study is designed to examine the ground water quality of Tehsil Barnala (AJK) to highlight all those factors which are detrimental for drinking

water quality. This study will provide a valuable data about the evaluation of ground water quality of Tehsil Barnala, as there is no previous study or complete published data on this issue.

## METHODOLOGY

### *Study area*

The study area selected for investigation of ground water quality is Tehsil Barnala, District Bhimber. District Bhimber is situated at the southern region, amongst ten districts of Azad Kashmir, Pakistan. Bhimber is bordered by Mirpur city to the northwest it has a distance of 50 km from Mirpur. It is only 166km from capital Islamabad and is divided into 3 teshsils (1) Bhimber (2) Samahni (3) Barnala.

Tehsil Barnala consists of both plains and hills It has climate similar to adjacent areas of Punjab. Summer is hot from April to October and winter proceeds from December to January, people are fortunate to enjoy all seasons. Water resources are well water, bore hole water, spring water and surface water. These resources are declining day by day because of inadequate management. Most of the population rely on ground water resources *i.e.*, bore hole water ,well water etc, for drinking and irrigation purposes.

### *Sampling and chemical analysis*

A total Of 7 ground water samples were collected from different localities of Tehsil Barnala. Ground water samples including well water, borehole water and tap water of water supply lines were collected according to the standard methods of American Public Health Association (APHA, 1998). Samples collection and transportation was according to standard methods of APHA (APHA, 1998). Samples were collected in plastic polyethylene bottles which are disinfected by nitric acid and pH was adjusted at 4. Samples were carried in the lab in ice cooler and they were transferred to the laboratory as early as possible. Ground water samples were analyzed for (selected) physical, chemical and biological parameters, namely temperature, pH, turbidity, color, odor, taste, electric conductivity (EC), hardness (total calcium + magnesium), chlorides, nitrites, ammonium ion, arsenic and *Escherichia coli* (present or absent basis).

These water quality determining parameters were analyzed in well established laboratory of Azad Kashmir Environmental Protection Agency

(EPA), Mirpur, Azad Kashmir. The results were compared with WHO guidelines suggested for drinking water quality.



Fig. 1. Showing map of District Bhimber, AK, and Pakistan.

## RESULTS

### *Physical parameters of ground water of District Bhimber*

#### *pH*

In Tehsil Barnala highest value of 8.5 was measured at Malot and lowest value was 7.4 in Moel. Results revealed that pH values were within prescribed limits of WHO in all water samples of Tehsil Barnala.

#### *Temperature, taste, color and odor*

Temperature of all sampling sites of Tehsil Barnala ranges from 33.3–33.8°C. Taste, color and odor of all water samples of district Barnala were unobjectionable.

#### *Turbidity*

In Tehsil Barnala turbidity range was from 0.02 – 0.15 NTU. Turbidity values were found according to recommended limits of WHO in all water samples

*Total dissolved solids and electric conductivity*

TDS value varies from 66 – 436 in water samples of Tehsil Barnala And values of EC lies between 132 to 872.

*Biological parameters of ground water of District Bhimber*

All ground water samples taken from Tehsil Barnala were contaminated with pathogenic microbes *i.e. E. coli*.

*Chemical parameters of ground water of District Bhimber**Total hardness*

In Tehsil Barnala maximum value of total hardness *i.e.* 107 was measured in water sample of Iftharabad and minimum value was 62 in Barnala.

*Ammonium ion*

In Tehsil Barnala mean value of ammonium was 0.02 mg/L in all water samples. Results exposed that values of ammonium were within prescribed limits of WHO in all water samples of District Barnala

*Phosphate*

In Tehsil Barnala phosphate values lies within 1.06 to 2.6 mg/L.

*Chloride ion*

In Tehsil Barnala values of chloride ion range from 10.5 to 39 mg/L in Kot Jaimel and Iftharabad, respectively.

*Nitrite*

In Tehsil Barnala lowest value of nitrite measured in water sample of Malot was 0.1 mg/L and highest value measured in water sample of Chhamb was 0.2 mg/L.

*Lead*

In Tehsil Barnala maximum value was 2.6 in Moel and minimum lead value, 0.02 mg/L was measured in ground water of Kadhala.

TABLE I.- CHEMICAL ANALYSIS OF GROUND WATER OF TEHSIL BARNALA.

Site names	Arsenic (mg/L)	Total hardness (Ca+Mg)	Ammonium ion (mg/L)	Phosphate (mg/L)	Chloride ion (mg/L)	Nitrite (mg/L)	Lead (mg/L)
LBKD	0	70	0.03	1.7	32.3	0.02	0.02
LBBN	0	62	0.02	1.8	35.2	0.02	0.09
LBKJ	0	70	0.03	2.6	10.2	0.03	0.12
HPMO	0	92.5	0.03	2.03	20.3	0.09	0.26
LBIF	0	107.5	0.01	1.06	39	0.11	0.18
HPCHB	0	95	0.05	1.4	31.6	0.2	0.19
HPML	0	100	0.05	1.7	37	0.01	0.09

TABLE II.- PHYSICAL AND BIOLOGICAL ANALYSIS OF GROUND WATER OF TEHSIL BARNALA.

Site name	Turbidity (NTU)	TDS (ppm)	EC ( $\mu$ S/cm)	Temp. ( $^{\circ}$ C)	pH	Color	<i>E. coli</i>	Taste & odor
LBKD	0.07	66	132	33.4	8.2	Colorless	Present	Un objectionable
LBBN	0.02	385	771	33.4	7.6	Colorless	Present	Un objectionable
LBKJ	0.07	416	832	33.6	7.6	Colorless	Present	Un objectionable
HPMO	0.03	352	705	33.3	7.4	Colorless	Present	Un objectionable
LBIF	0.11	436	872	33.3	7.4	Colorless	Present	Un objectionable
HPCH	0.15	420	840	33.4	7.6	Colorless	Present	Un objectionable
HPML	0.12	112	224	33.3	8.5	Colorless	Present	Un objectionable

Tehsil Barnala; HP, Hand Pump, LB: Local Bore, WL: Well, KD: Kadhala, BN: Barnala, KJ: Kot Jaimel, MO: Moel, IF: Iftikharabad, CH: Chhamb, ML: Malot.

## DISCUSSION

After a detailed analysis of ground water of Tehsil Barnala, it is discovered that some physical water quality determining parameters like pH, turbidity and temperature values were within prescribed limits of WHO in all water samples of Tehsil Bhimber. Color, taste and odor of all samples had not any variation from prescribed limits.

Chloride ion concentration was found below the recommended limits. Total hardness ( $\text{Ca}^{+} + \text{Mg}^{+}$ ) values in a few water sites were lower than prescribed limit. Value of lead was found problematic in one water sample of Tehsil Barnala.

Arsenic was not detected in any water sample of Tehsil Barnala and ammonium concentration in ground water was found satisfactory, its ratio is not hazardous in any area of Tehsil Barnala. Phosphate values were also found within the prescribed limits. No deviation from suitable value was observed in any water sample of Tehsil Barnala. Concentration of nitrite was much higher in ground water sample of Chamb site, that reflects the incorporation of civic and domestic waste water into the drinking water resources, as improper designing of waste water drains, septic etc. was observed in the Chamb Town, and due to ill planning of civil work/ drainage system, drinking water resources are receiving the high concentration of nitrite.

Biological contamination was present in almost all water samples of Tehsil Barna which indicated that there is certain chances of addition of fecal matter with ground water. Due to which prevalence of water born diseases is common in the area. Diseases like diarrhea, gastroenteritis, shigellosis and flu are common.

### CONCLUSIONS AND RECOMMENDATIONS

It is concluded that ground water of Tehsil Barnala is not suitable for drinking without treatment as it is disgustingly infected with pathogenic micro organisms like *E. coli* and fecal coliform. Besides it, values of some physicochemical water quality determining parameters are also beyond the recommended limits of WHO. Results have proven that consumption of unsafe drinking water is one of the major cause of prevalence of water born diseases like diarrhea, typhoid fever and malaria etc in the study area. Following measures can be adopted to decontaminate the water so that water born infections can be controlled:

1. Water should be disinfected by water supply agencies before its distribution to rural and urban communities. To avoid additional contaminants to the water, the physical infrastructure that is used for distribution of water to the communities must be made from suitable materials so that accidental contamination does not occur.



2. Water holding and supply tanks should be efficiently cleaned and disinfected for algal and fungal growth on walls of tanks.
3. In case of private water sources such as well and bore hole it is suggested that drinking water should be pumped directly from its source so that risk of additional contamination from water holding tanks can be minimized.
4. Waste material discharged from domestic and industrial sources should be efficiently disposed and sewage drains should be properly covered. Utilization of polluted water for cultivation of crops should be avoided as it cause potential hazards to consumer's health.
5. At house hold level water contamination can be avoided by proper storage of water in clean utensils and boiling of water before consumption particularly for drinking purpose. Boiling of water is the traditional way of disinfecting water, it kills a number of pathogenic micro organisms, but boiling should not be considered enough because it cannot reduce the chemical pollution and cannot kill thermopiles.

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**PSEUDOMONAS AEUROGINOSA AND STREPTOCOCCUS  
HEMOLYTICUM IN THE LARVA OF GOAT WARBLE FLY  
PRZHEVALSKIANA SILENUS (BRAUER), A UNIQUE COMBINATION:  
FACULTATIVE OR COMMENSALS**

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**Abstract.-** *Pseudomonas aeuroginosa* and *Streptococcus hemolyticum* were isolated and observed inside hundred larvae of *P. silenus brauer* of Rohila goat breed of Dera Ghazi Khan in December, 1997. The study was unique in its nature to explore the facultative or commensal bacterial help of larva of goat warble during its L2 migration or its L3 sojourn.

**Key words:** *Pseudomonas aeuroginosa*, *Streptococcus hemolyticum*, *P. silenus brauer*, Rohila goat breed, Warble fly.

## INTRODUCTION

Goat warble/Hypodermatitis is wide spread disease in Iran, Italy, Greece and Pakistan as reported by Himonas *et al.* (1982), Giangaspero *et al.* (1996), Rahbari and Ghasemi (1997) and Ayaz (1998). Warbles fly not only annoys the animals but also reduces its weight (Liakos, 1986) and milk (Solusby, 1982). Warble fly is renowned as deleterious pest in leather and tanneries industry. There are various examples of host-parasite relationship. *Pseudomonas aeuroginosa* is the one of the fastidious bacteria which is known for its hard life and cosmopolitical distribution. It is present in hospitals, laboratories and various other places of contamination. It is treated with difficulty due to its resistance to the most of antibiotics. *P. aeuroginosa* and *Streptococcus hemolyticum* is not common inhabitant of larvae of goat warble fly. Presence of *P. aeuroginosa* and *S. hemolyticum* will open new vistas for control of warble fly infestation and

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disclose more secrets of nature. This study is unique in nature to explore the role of various bacteria present inside the goat warble fly larva in Pakistan.

## MATERIALS AND METHODS

A 100 L2 and L3 larvae of *Przhevalskiana silenus* (Brauer) were collected from the ten goats of Rohila breed (Hairy goat breed) by gentle pressing method. Goats were appearing healthy but larvae were diagnosed through palpation method during the month of December 1997; brought from nearby area of Tommi, District Dera Ghazi Khan. External surface of the larvae were cleansed thoroughly with 0.5% formaldehyde and 75% alcohol to eliminate the chances of microbial infection/ contamination. Later on larvae of goat warble fly were dissected longitudinally under the stereo dissecting microscope. The internal contents of larvae were taken aseptically and diluted in distilled water. Material diluted in distilled water was spreaded on slide for Gram stains method to identify the characters of microbes under higher magnifications of oil immersion lens and diluted material was also poured on the Petri dish containing nutrient agar and blood agar to observe the growth and colony characteristics of microbes if any. Nutrient media and blood agar was prepared under aseptic conditions. Nutrient media and blood agar was placed in an incubator at 25°C for 48 hours for incubation.

## RESULTS

The slides were observed under high magnifications oil immersion lens. Grams stain showed presence of gram negative, rod shaped, scattered bacteria showing similarity with size of *P. aeuroginosa*. Gram negative rod shaped microbes were in abundance and very little numbers of gram positive Streptococci were present on the slide.

### *Growth on nutrient agar and blood agar*

After 48 hours of incubation two types of colonies were observed in Petri dish. There were white foci, round in nature. They were scattered like leaf superficially on the media and abundance on area.

## DISCUSSION

Third stage larvae (L3) of goat warble fly *P. silenus* (Brauer) always

damage the skin and make hole. They respire from the posterior spiracles after boring on the back of the animal. Presence of *S. hemolyticum* has been observed earlier by Norman and Younger (1979) that may activate the prompt expulsion process of L3 larvae through the production of pus from blood, serum and tissue of the body present in the hole or sack made by larvae. The isolation of bacteria like *E. coli*, *S. pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermis* and *Klebsiella pneumoniae* have been reported but externally in cattle grub in Iran by Tavassoli *et al.* (2010). *S. hemolyticum* may get entry into the gut of larvae during the feeding of tissues contents like the blood and serum of the goat or host after making the hole through skin. *P. aeuroginosa* is common commensal of the wounds that sometimes may aggravate the conditions to the host but may facilitate for larva. They might have got entry into L3 larvae through the feeding process from the tissues or during its L1 stage of first entry into the host body, after hatching of eggs. The L1 larvae of *P. silenae* (Brauer) may contaminate themselves from the superficial layers of goat skin/ dermis. The enumerable quantity shows that they got multiplication inside the larvae during its sojourn in the body of host. The presence of *P. aeuroginosa* inside the larvae and in the wound, through its excretion may delay the healing process of the wound created by warble fly larvae on the skin. The purpose of this study was to observe the bacterial contamination inside the larvae of goat warble fly to avoid the further complication confronted with the treatment and control of the warble fly menace.

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## EARTHWORMS — THE SOIL ECOSYSTEM ENGINEERS

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**Abstract:-** In agricultural systems, earthworms are important regulators of decomposition, nutrient cycling, soil organic matter dynamics and pathways of water infiltration as a result of their feeding, casting and burrowing activities. Abundance of earthworms in soil promotes plant growth and enhance crop yield. The utilization of earthworms for vermicomposting is a potential breakthrough in soil biology sciences. Earthworms as a natural resource in agriculture need to be conserved and managed on sustainable bases, employing necessary farm management practices. Indiscriminate use of pesticides in cotton, potatoes, vegetables and fruit crops has adversely affected the earthworm biodiversity. Pesticides affected earthworms are the common prey of birds and small mammals, and thus play a key role in the biomagnification process of several soil pollutants and contaminants. Thus, incorporating ecological concepts into the design of farming systems and practices is highly desired.

**Key words:** Cotton fields, earthworms, pesticide impacts, vermicompost.

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## INTRODUCTION

Earthworms are dynamic members of soil biodiversity which facilitate the recycling of crop residues and organic matter throughout the soil profile (Scheu, 1987; Zhang and Hendrix, 1995). Statements in the literature such as “Sacred animals” by Cleopatra and “Intestines of Earth” by Aristotle emphasize the importance of earthworms as ecological service providers. It is also stated that earthworms have been around for 120 million years on the earth planet. More than 80% of the biomass of terrestrial invertebrates consists of earthworms. Charles Darwin’s (1881) famous book, “The Formation of Vegetable Mould through the Action of Worms”, provides a good example of an alarming publication explosion wherein he described earthworms as beneficent gardeners and industrious ploughmen, after studying them for more than 40 years. Also, he said; “worms seem to be the great promoters of vegetation, perforating and loosening the soil, rendering it pervious to rains and the fibers of plants by drawing straws and stalks of leaves and twigs into it, and most of all, by through up such infinite numbers of lumps of earth called worm casts, which being their excrement, is a fine manure for grain and grass”. He estimated that the annual production of worm’s casts in English pastures was 18.7 to 40.3 tons per hectare, which can be considered as equivalent to 5 mm deep soil being deposited annually. In India, Krishnamoorthy (1985a) reported 133 and 99 tons dry weight of earthworm casts/ha in grass and woodland experimental sites near Bangalore. In the absence of a rich soil biodiversity, 500-1000 years may be required to create 2.5 cm of top soil and that under favourable conditions a task force of earthworms can do the same job in 5 years (Barrett, 1955).

It has been reported that 3,627 earthworm species had been described worldwide, with an annual average addition of 68 species (Lee, 1985; Reynolds, 1994). Lavelle *et al.* (1998) developed a database of 457 earthworm’s species from tropical agro ecosystems of 28 countries with detailed geographical, environmental and socio economics of the sites of the collection. The overall richness is expected to be at least twice this value, with most species still unknown from the tropics. Only half a dozen or more are being considered important or utilized in agriculture. They range in size from a fraction of a centimeter to two or more meters long, and comprise from surface dwelling (epigeic) species to deep burrowing ones (endogeic). There is wide range of variations in their biology, behaviour, food and feeding habits and environmental requirements. Unless these are investigated and understood, their use in vermiculture and vermicomposting is a difficult exercise to work out.

The main purpose of this article is to create awareness among the stakeholders and promote earthworm biotechnology in the country as earthworms have relevance in the present day context of natural/organic/permaculture farming. Earthworms, though well studied soil organisms all over the world, are badly neglected in Pakistan. Even the work on systematic and taxonomy is incomplete. Up till now, no suitable species of earthworm has been found which can be utilized for vermicomposting.

### **BENEFITS OF EARTHWORMS**

#### *Improvement of soil structure*

Soil improvement is the major benefit of values of conserving/maintaining earthworm populations in the cultivated and pasture lands. In fact they are master soil builders and according to Aristotle are the intestine of earth, and are extremely important in the soil formation. In both natural and agricultural systems earthworms are important regulators of decomposition, nutrient cycling, soil organic matter dynamics and pathways of water movement as a consequence of their feeding and burrowing activities. As soil ecosystem engineers they influence the distribution of organic matter in the soil profile and improve physical structure of the soil. They reduce soil compaction and thatch accumulation. These organisms, therefore, should be properly managed and conserved through sustainable farm management practices. The number of earthworms under long-term (25 years) zero-tillage and conventional tillage in a dry land wheat-fallow rotation were studied by Clapperton *et al.* (1997). The results showed that there were significantly more earthworms in zero-tillage than under conventional tillage. Long-term experiments have shown that there were more earthworms in plots treated with organic fertilizers than in plots treated with inorganic fertilizers (Edwards and Lofty, 1982).

#### *Influence on chemical properties of soil*

Earthworms, besides physical, also influence chemical properties of the soils (Edwards and Lofty, 1982). It has been observed that earthworm casting contained elevated amounts of  $\text{NH}_4$ ,  $\text{NO}_3$ , Mg, K and P relative to bulk soil (Gupta and Sakal, 1967; Syers *et al.*, 1979; Tiwari *et al.*, 1989). In the castings, ammonia and urea can make upto maximum of 48% to 60% of the total nitrogenous products (Needham, 1957). Krishnamoorthy (1985b) studied the nitrogen contribution by earthworms which showed that about 472-443 kg N/ha annually is being contributed to the soil. The mucous secretions from the body of earthworm contain high concentrations of organic N and ammonium (Needham,



1957) and may serve as a substrate for fungi and bacteria (Edwards and Fletcher, 1988). Both the casts and mucous secretions enhance the level of nitrogen and this in turn leads to the lowering of C/N ratio (Senapati *et al.*, 1980). Recent studies in U.S.A. showed that the fresh casts of earthworms are five times richer in phosphates, eleven times richer in available potash, two times more magnesium and calcium than in the upper 15 cm of soil. Also, earthworms are being studied as one way to measure carbon sequestration, the capture and use of carbon in the soil.

#### *Effects on plant growth and yields*

About 30 earthworm species have been tested for their effects on plant production. The results have shown that earthworms cause beneficial effects on plant growth. Earthworms increased plant production in most cases (72% of the results were positive), and the overall average increase obtained was  $56 \pm 9\%$  (S.E) for shoot mass, and  $36 \pm 9\%$  for grain production (Lavelle *et al.*, 1998). The earthworm benefits appear to be particularly important in perennial cropping systems.

#### *Role in low-input agricultural systems*

Earthworms play an important role in low-input agricultural systems. Their burrowing and casting activity involves upto 11.6% of the organic carbon and 12.9% of the total nitrogen of the 0 to 15 cm top soil in undisturbed or recovering system. Earthworms have been extensively used to restore saline and degraded lands (Ansari and Ismail, 2008). For this purpose deep burrowing species of earthworms are being recommended. In 1954, sea water flooded the lands in Holland and was restored to cultivation by inoculating earthworms in soil. For this purpose thousands of tons of earthworms were imported from U.S.A. and aerially broadcasted on the affected lands. The importance of earthworms grows for 'no-till' farming, a concept widely and seriously pursued in sustainable agriculture. Majority of the no-till does not work without earthworms. No-till farming can create a hazardous situation if it leads to the build up of agro-chemical residues on the soil surface and may prove toxic to earthworms.

#### *Role as predators*

Earthworms are predators on parasitic nematodes and pathogenic fungi (Senapati, 1992). Their use in vegetables crops such as potatoes, chillies and tomatoes will be highly useful for enhanced productivity by preventing nematode damage.

*Pharmaceutical uses*

Earthworms are being used since ancient times for the treatment of the illness such as bladder stones, jaundice, rheumatism, toothaches, to cure mums and measles, fever, impotency etc. Earthworms can help dissolve blood clots for stroke patients.

*Earthworms as productivity and environmental indicators*

Earthworms are excellent indicators of soil health and productivity. By studying the organic contents of earthworm casts, scientists in the ARS Soil and Water Management Research Unit at the University of Minnesota have discovered a simple way to determine soil health in agricultural fields. In Australia, earthworms have been recommended as the most suitable indicator for monitoring sustainability in agriculture (Bucherfield *et al.*, 1997). Also, the earthworms are excellent environmental indicators of the adverse impacts of pesticides, pollutants and contaminants. Earthworms are an ideal organism for use in the study of environmental contamination, both as an indicator species and a test species. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil health. The suitability of earthworms as bioindicators in soil toxicity is largely due to the fact that they ingest large quantity of the decomposed litter, manure, and other organic matter deposited on the surface of the soil. Earthworm's skin is a significant route of contaminant uptake and thus investigation of earthworm biomarkers in the ecological risk assessment can be helpful.

**EARTHWORM BIOTECHNOLOGY**

Earthworm biotechnology is the combination of vermiculture and vermicomposting, i.e. application of earthworms for development of arable soils, restoring saline soils, breakdown of organic/plant materials, aeration, drainage, for maintaining environment quality and monitoring soil fertility. This technology is both environmentally sustainable and economically feasible and socially acceptable.

*Vermiculture*

It is generally referred to the development of mass earthworm culture for various uses. In other words, vermiculture means scientific way of breeding and

raising earthworms under controlled conditions, both in-door and out-door. It aims at multiplying earthworms from breeder stock in shortest possible time. Some species of earthworms such as *Eisenia foetida* (red worm), *Eudrilus eugeniae* (night crawler) and *Perionyx excavatus* are being used in earthworm biotechnology. *E. foetida* is world's most widely used earthworm for vermicomposting because of fast breeding potential and that they need very little care. This species is capable to tolerate wide fluctuations of temperature and humidity. Also they can feed on wide variety of degraded and decomposable organic wastes. *E. eugeniae* is also, fast breeding species and second most widely used and recommended in earthworm biotechnology. The third species is also, commonly used for vermiculture and vermicomposting in India. Its reproduction rate is double that most earthworm species commonly used in vermiculture.

### *Vermicomposting*

It is a rapid process of composting organic matter (animal and plant wastes) with earthworms, ending up with a rich end product called casting and vermicompost - high value organic fertilizer. The vermicompost contains everything the plant needs - nitrates, phosphates and potash in abundance and readily available to plants in just the condition in which plants need them. Beside this some essential micronutrients and beneficial microbes are the parts of vermicompost.

TABLE I.- CHEMICAL AND MICROBIAL ANALYSIS OF VERMICOMPOST SAMPLES PRODUCED AT NARC, ISLAMABAD.

Chemical Nutrients	Value*	Microbes	Value**
Total N	1.25%	1. Bacteria	$5.6 \times 10^6$
Total P	0.45%	2. Bacteria	$6.7 \times 10^6$
Total K	0.60%	3. Bacteria	$2.9 \times 10^6$
Available Na	0.29%	<u>Average</u>	$5.1 \times 10^6$
Available K	0.29%	1. Actinomycetes	$3.1 \times 10^5$
Available P	10 mg Kg <sup>-1</sup>	2. Actinomycetes	$7.3 \times 10^5$
Total Zn	112 mg Kg <sup>-1</sup>	3. Actinomycetes	$6.8 \times 10^5$
Total Cu	20 mg Kg <sup>-1</sup>	<u>Average</u>	$5.7 \times 10^5$
Total Fe	3860 mg Kg <sup>-1</sup>	1. Fungi	$2.5 \times 10^3$
Total Mn	391 mg Kg <sup>-1</sup>	2. Fungi	$4.6 \times 10^3$
Total Oc	18.03%	3. Fungi	$8.3 \times 10^3$
C/N	14.42	<u>Average</u>	$5.1 \times 10^3$
pH(1:2)	7.02	—	—

\*Based on dry weights except pH

\*\*Cell counts per gram of vermicompost

A modest activity on vermiculture and vermicomposting was initiated by the author of this article at National Agriculture Research Centre (NARC), which at present is being continued by the scientists of Soil Biology Laboratory of Land Resources Research Institute. Recently, Pakistan Agricultural Research Council has established a Vermiculture and Vermicomposting Research Unit at NARC under Research for Agricultural Development Program (RADP). The results of chemical and microbial analysis of the vermicompost produced at NARC are presented in Table I and its comparison with FYM (Table II).

Though the NPK value of vermicompost is lower than any standard chemical fertilizer, several experiments have proved that worm casts can promote lush growth of plants. This may be due to plant growth promoters like cytokinins and auxins present in the casts. Vermicompost can further be enriched by adding some biofertilizers such as *Acetobacter* and phospho-bacterium. An antagonistic fungus, *Trichoderma viride* is also added to the compost to help in preventing the incidence of some fungal diseases.

TABLE II.- COMPARATIVE NUTRIENT PROFILE OF VERMICOMPOST AND FARM YARD MANURE (FYM).

Nutrient	Vermicompost	Farm Yard Manure
N (%)	1.6	0.5
PO (%)	0.7	0.2
KO (%)	0.8	0.5
Ca (%)	0.5	0.9
Mg (%)	0.2	0.2
Fe (ppm)	175.0	146.5
Mn (ppm)	96.5	69.0
Zn(ppm)	24.5	14.5
Cu(ppm)	5.0	2.8
C:N Ratio	15.5	31.3

The use of vermicompost promotes plant growth, improves soil health and enhances productivity. Pot studies in green house on vegetable production with NPK, sheep manure and vermicompost have indicated more yields with the application of vermicompost than with NPK and manure (Saciragie and Dzelilovic, 1988). It has been recorded that using worm casts as manure of ornamental plants like Salvia, Asters and Violets improved vegetative growth and flowering as compared to plants grown on FYM. The application of vermicompost in a paddy field enhanced the activity of mycorrhizae (N-fixer)

and actinomycetes. Some reports suggest that with the use of earthworm compost, a maximum increase of 18% and 12% in the maize and stalk production, respectively, has been recorded over three crop cycles.

Vermicompost use in areas of value added sectors such as floriculture and horticulture needs to be encouraged and promoted, specifically in tunnel farming. Vermicompost use should be adopted as one of the most important key component of organic/natural farming. Organic fertilizers favor earthworms more than mineral fertilizers (Khan, 2012).

Vermicomposting, at any scale, may have an important role to play in urban and rural waste management. Civic bodies in many countries have adopted this way of waste management. The scope of commercialization is wide open because market size is very big. In India, vermiculture and vermicomposting have been established as industry and tremendous research work on earthworm is under way in many universities and research institutes. Many farmers in India, have become entrepreneurs in vermicomposting.

### **EARTHWORM RESEARCH IN PAKISTAN**

As early as 1916, Prashad identified 6 new species of earthworms collected from grassy lawns of parks and some adjoining areas of Lahore. After a pause of more than four decades Bhatti (1962) found 14 new species of earthworms collected from various localities in Lahore city Corporation areas. These were the only two taxonomic studies which so far have been conducted in Pakistan. Starting from 1980's some studies (MSc. Thesis work) on morphometrics, distribution, abundance, growth and reproduction have been conducted (Table III). Some new species of earthworms from Pakistan have been described by different authors. All these have been reported from Punjab while from Sindh, KPK and Balochistan knowledge on earthworms is lacking. Studies on impacts of pesticides on earthworms, biodiversity, vermiculture and vermicomposting are yet to be attempted.

Some field studies were conducted by Khan (2000) in Quetta, Hanna Lake, Mustung, Lora Lai, Kallat, Gilgit, Islamabad, Attock, Fatehjang and Multan to evaluate the effect of different climatic parameters on the growth and reproduction of manure worms (*E. foetida*). Extreme limits of temperature adversely affected growth at Kallat, Multan and Gilgit, while at other sites the vermiculture progressed normally. These preliminary studies indicated that vermicomposting activities can be imitated in these areas.

TABLE III.- SOME STUDIES CONDUCTED ON EARTHWORMS IN PAKISTAN

Author	Year	Location	Remarks
Prashad, B.	1916	Lahore	06 new spp.
Stephenson, J.	1923	G.C. Lahore	compiled Oligochaeta, Fauna of British India
Bhatti, H.K.	1962	Lahore	14 new spp.
Elahi, S.N.S	1981	Faisalabad	morphometric, abundance
Ghafoor, A. <i>et al.</i>	1988	Faisalabad Division	no new species
Ghafoor, A. <i>et al.</i>	1989	Faisalabad Division	population fluctuation
Noreen, U.	1997	Faisalabad	abundance and ecology
Ghafoor & Qureshi	1999	Faisalabad	05 (new records)
Rana, S.A. <i>et al.</i>	2000	Islamabad	02 new spp.
Rana, S.A. <i>et al.</i>	2000	Faisalabad	03 new spp.
Qureshi <i>et al.</i>	2000	Faisalabad Areas	habitat preference
Rana, S.A. <i>et al.</i>	2000	Faisalabad Division	eco- distribution
Ghafoor <i>et al.</i>	2003	Attock District	02 new spp.
Nosheen, A.	2004	PMAS, AAUR	reproduction & growth
Siddique <i>et al.</i>	2005	PMAS, AAUR	reproduction & growth
Nadeem, A. <i>et al.</i>	2007	Faisalabad Orchards	habitat preference
Fatima & Rana	2007	Faisalabad Wheat Fields	morphometric variations
Rana, S.A. <i>et al.</i>	2007	Faisalabad, Citrus Orchards	methyl parathion effect
Rana, S.A. <i>et al.</i>	2008	Sugarcane Fields	genetic diversity
Khan, A.A	2012	Islamabad	Vermicompost analysis
Andleeb, S	2012	AJ&K	01 new spp. (pers. commu)

### IMPACT OF PESTICIDES ON EARTHWORMS

Damage to the crops and food products, generally, are estimated at between 25% and 50% in the agriculture sector of the world. Chemical control of pests is the quickest way of controlling them, against cultural and biological practices which require longer span of time. However, there are serious ecological, economic and environmental problems with the use of pesticides. Persistence and bio-accumulation of pesticides is one of the serious problems in the ecological web. Pesticide load in the soil and on its surface leads to the destruction of beneficial biological species (*e.g.* earthworms), and through this process imbalance in the ecosystem is created.

Of the imported quantity of pesticides in Pakistan, more than 70% of this is used to control insect pests of cotton. The growth in pesticide use in Pakistan, during the last three decades, grew as much 20-30% each year. Indiscriminate

(un-regulated) use of pesticides on cotton crop has heavily polluted with residues the cotton belts of Punjab and Sindh provinces where 7-10 sprays of pesticides are commonly conducted. Pesticide use has been seen both by farmers and governments as an effective method with clear criteria and practices remaining under the farmer's control. However, in the real situation majority of the farmers are functional illiterates and cannot read the pesticide label and believe in the "rock ém, sock ém, knock ém dead".

In Pakistan, little information exists to make even a conservative estimate of the populations or species of earthworms and other soil biodiversity components that are adversely affected by pesticides. Results of some studies in the cotton fields, citrus orchards and laboratory tests conducted in Pakistan are being reported in this article (Rana *et al.*, 2007, 2008, Khan, 2012)

#### *Mechanisms of exposure to the pesticides*

Concern regarding the impact of pesticides on the soil biota has increased in recent years. Among these, earthworms are exposed to agro-chemicals in crop fields and fruit orchards in a variety of ways as follows:

- When the soil is treated with pesticides to control soil-borne pests, the chemicals get mixed with soil so that the pest comes into maximum contact with the pesticides. During this process earthworms are also exposed to pesticides.
- Earthworms are exposed to surface residue of pesticides which are sprayed on field crops (*e.g.*, cotton). Earthworms that move over such contaminated soil surface particularly *Lumbricus terrestris* and *Pheretima spp.*, can pick large quantities of toxic chemicals. Repeated multiple sprays can lead to a gradual build-up of pesticide residues on the soil surface and into the sub-surface strata. In such cases the chronic impacts are deleterious on earthworm populations. It has been estimated that more than 90% of the pesticides used for pest control goes directly to soil.
- The practice of no-till or zero tillage can create a hazardous situation when repeated treatments of pesticides are made.

#### *Factors influencing exposure*

The level of exposure of earthworms to pesticides depends upon multiple variable factors, such as the soil type, the environmental conditions, and the behaviour of different earthworm species. Pesticide properties that affect their

impact upon earthworms include water solubility, volatility, lipid/water partition co-efficient, adsorptive capability and their persistence in the soil.

#### *Toxicity of pesticides to earthworms*

In current use, a number of insecticides, herbicides and fungicides are toxic to earthworms. They are either highly toxic or moderately toxic, and some have no adverse effects on earthworms (Roberts and Dorough, 1985; Edwards and Bohlen, 1992; Mostert *et al.*, 2000). Mortality has been the most frequently used and rapid parameter to evaluate chemical toxicity in earthworms. Among all the pesticides, carbamates are highly toxic to earthworms while majority of pyrethroids have no adverse effects. An interesting aspect of use of benomyl to control apple scab in orchards is that this disease is transmitted from year to year in fallen leaf litter. A good earthworm population will remove most of the fallen leaves in autumn and winter but earthworms are susceptible to benomyl residues and thus the natural control agent is destroyed. Organochlorines affect earthworm populations at all levels of toxicity. Majority of contact and fumigant fungicides/nematicides, and herbicides are highly toxic to earthworms. Among fungicides benomyl and carbendazim are highly toxic while majority of them have no adverse effects. Organophosphates are moderately toxic or having no adverse effects on earthworms. Very few herbicides are toxic to earthworms while majority of these are slightly toxic (Table IV). Chronic or sub lethal toxicity of pesticides has wider range of impacts on earthworms because of bio-accumulation. During this process, the growth, reproduction, and immune systems of earthworms are effected (Yasmin and D'Souza, 2010; Bunn *et al.*, 1996).

#### *Experiences in Pakistan*

The acute and chronic sub-lethal effects of agricultural pesticides have little been studied in Pakistan. Studies conducted by Eco-Toxicology Research Laboratory, NARC, Islamabad have indicated that organophosphate compounds such as endosulfan, thiodan, methamidophos, cypermethrin are highly toxic to *L. terrestris*, a common and widely distributed earthworm species in arable lands in Punjab (Khan, 1999). Anwar *et al.* (2014) monitored residues of organophosphate and organochlorine pesticides in cotton growing areas in Bahawalpur district and found heavy residues load of dichlorvos, dimethoate, methyl parathion, endosulfan, fenitrothion, mevinphos, chlorpyrifos and profenofos. Another study conducted in citrus orchards at University of



Agriculture, Faisalabad showed severe toxic effects of methyl parathion to seven species of the genus *Pheretima* (Rana *et al.*, 2007). Deep burrowing species such as *Apporectodea caliginosa*, *A. longa* and *Octochaetoides beatrix* had no adverse effects.

TABLE IV.- IMPACTS OF PESTICIDES ON EARTHWORMS

Pesticide group	Highly toxic	Low toxicity	Slightly/ Moderate toxicity	No adverse effects
Carbamates	Aldicarb, Benomyl, Carbaryl, Methomyl, Propoxur, Thio- fanox, etc	-	-	-
Pyrethroids	-	-	-	Majority
Contact and fumigant, fungicides/ nematicides	Methyl bromide, Methomyl, Chloropicrin	-	-	-
Fungicides	Benomyl, Carbendazin	-	-	Majority
Herbicides	Very few	-	Moderate	-
Organochlorines	Endrin, Chlordane	BHC, Aldrin, Dieldrin	Endosulfan, Heptachlor, Isobenzam	DDT (with normal application rate)
Oorganophosphates	-	-	Parathion, Disulfoton, Dyfonate	Diazinon, Malathion, Dursban, Menazon, Fenitrothion

To assess the environmental risk of the use of pesticides in cotton crop on earthworms, a field study was conducted, by the author of this article, in five districts of Punjab. During the months of November and December, 1999; 72 fields of cotton and 18 fields of wheat previously used for cotton, were sampled in Multan, Vehari, Lodhran, Khanewal and Faisalabad. From each field three

samples were taken by placing a steel made quadrat (50x50x15cm). Each quadrat was filled with 0.025% water dilution of formalin. After about 20-30 minutes the earthworms appeared on the inside surface area of the quadrat. A total of 270 quadrats were taken. One hundred and eighty nine were examined in cotton fields while 54 in wheat and rest were along water courses.

The sampling results are summarized in the Table V, which indicated that 97.04% of quadrats were found negative while only eight quadrats were found positive for earthworm in cotton which was grown in orchards (mango and citrus). Information gathered from farmers indicated that mostly polytrin-C; azodrin; navacron; fos-tec; somisidon; talstar; sandaphos and karatay were used on cotton. The number of applications ranged from 4-10 with an average of seven. These results indicate that insecticides have severely affected the populations of earthworms in cotton growing areas of Punjab. Surprisingly, the earthworms were absent along the water courses which is normally the selective habitat of earthworms. Old farmers when interviewed for past information on earthworms, majority of them reported abundance of earthworms in cotton fields when no or little pesticides were used on cotton. In orchards where eight samples were found positive, the earthworms may have survived around the tree trunks where organic manure cover was sufficient and that the pesticide residues have not reached there. This study has indicated that how adversely the pesticides have affected the earthworm biodiversity in cotton fields. Therefore, it is highly desired that availability of data on impacts of pesticides on earthworms be made mandatory for the registration of pesticides. Pesticides will remain effective tool for pest control but the challenge now is to find ways to use them judiciously, coupled with IPM technologies and best farm management practices to avoid many of the environmental hazards including the damage they cause to soil biodiversity of earthworms.

### **A CHALLENGE**

Soil management has greatly lagged behind crop management in terms of application of biological principles and methods. The challenge now arises for the soil biologist: where soil science knowledge is sufficiently advanced that a comparable impact can be made on the productivity and sustainability of agricultural systems through the manipulation of soil biological diversity and processes. The economic and social value of soil biodiversity should, however, be a significant component of the assessment of agricultural sustainability. Any agricultural system under going intensification, large numbers of farmers have

Table V

limited access to inputs, and therefore the maintenance and enhancement of soil biodiversity may be particularly relevant to such farmers. The economic and social value of soil biodiversity should, however, be significant component of the assessment of the agricultural sustainability. The university research systems can significantly contribute in this area of soil science.

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TABLE V.- RESULTS OF SAMPLING OF EARTHWORM POPULATIONS IN COTTON GROWING AREAS OF PUNJAB, PAKISTAN.

District/ Tehsil	No. of fields sampled	No. of quadrate examined	No. of quadrate examined in								No. sprays of insecticides
			Cotton		Wheat*		Along water courses		Cotton in gardens		
			No.	+ve	No.	+ve	No.	+ve	No.	+ve	
Multan	30	90	54	--	18	--	9	--	9	6	6-8(7)
Vehari	27	81	36	--	9	--	9	--	27	2	6-10(8)
Lodhran	09	27	09	--	9	--	--	--	9	--	6-8(7)
Khanewal	06	18	09	--	9	--	--	--	--	--	8-8(8)
Fasilabad	18	54	36	--	9	--	9	--	--	--	4-10(7)

## **Some Abstracts**

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## SECTION - I

### CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY

#### 1. BIOCHEMISTRY

##### STUDY ON QUALITY CHARACTERISTICS OF MILK OBTAINED FROM BUFFALOES AT LIVESTOCK MARKETS/PIRIES

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Present investigation was carried out at the Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam to evaluate the milk of buffaloes assembled for sale at livestock markets/piries during the year 2011-12. A total of three popular livestock markets/piries viz Mirpurkhas (MPKP), Tando Allahyar (TAYP) and Chambar (CP) were selected, and from each livestock markets/piries, a total of twenty (n=20) milk samples of buffaloes were collected. Parallel to these, a total of twenty (20) whole buffalo milk from dairy farms as control were also collected from the surrounding areas of Mirpurkhas and Tando Allahyar for comparison purpose. Physico-chemical characteristics such as titratable acidity, pH values, specific gravity, moisture, protein, fat, lactose, and ash and solids not fat contents were evaluated. Titratable acidity of milk of buffaloes brought for sale at MPKP ( $0.20 \pm 0.005\%$ ), TAYP ( $0.20 \pm 0.004\%$ ) and CP ( $0.20 \pm 0.005\%$ ) was significantly higher ( $P < 0.05$ ) than that of control milk ( $0.19 \pm 0.003\%$ ) and pH values of milk of buffaloes at MPKP ( $6.49 \pm 0.03$ ), TAYP ( $6.55 \pm 0.02$ ) and CP ( $6.52 \pm 0.02$ ) were relatively similar ( $P > 0.05$ ), but significantly ( $P < 0.05$ ) lower than that of control milk ( $6.63 \pm 0.01$ ). The specific gravity of milk of buffaloes at MPKP ( $1.035 \pm 0.0003$ ), TAYP ( $1.034 \pm 0.0003$ ) and CP ( $1.034 \pm 0.0002$ ) were relatively similar ( $P > 0.05$ ) and significantly ( $P < 0.05$ ) higher than that of control milk ( $1.033 \pm 0.0002$ ). The average moisture content of buffalo milk at MPKP ( $81.77 \pm 0.12\%$ ), TAYP ( $82.11 \pm 0.17\%$ ) and CP ( $81.80 \pm 0.12\%$ ) were relatively similar ( $P > 0.05$ ) but significantly ( $P < 0.05$ ) lower than that of control milk ( $83.54 \pm 0.048$ ). The fat content of milk of buffaloes at MPKP ( $7.76 \pm 0.059\%$ ), TAYP ( $7.83 \pm 0.050\%$ ) and CP ( $7.81 \pm 0.055\%$ ) was relatively similar ( $P > 0.05$ ) but significantly ( $P < 0.5$ ) higher than that of control milk ( $6.39 \pm 0.034\%$ ). The protein content of milk of buffaloes at MPKP ( $5.93 \pm 0.13\%$ ), TAYP ( $6.11 \pm 0.11\%$ ) and CP ( $6.02 \pm 0.12\%$ ) was relatively similar ( $P > 0.05$ ) but significantly ( $P < 0.05$ ) higher than that of control milk ( $4.63 \pm 0.08\%$ ). The lactose content of control milk ( $4.7 \pm 0.10\%$ ) was significantly higher than that of milk at MPKP ( $3.76 \pm 0.18\%$ ), TAYP ( $3.18 \pm$

0.15%) and CP ( $3.58 \pm 0.16\%$ ). The average ash content of milk of buffaloes at MPKP ( $0.77 \pm 0.024\%$ ), TAYP ( $0.75 \pm 0.019\%$ ), CP ( $0.75 \pm 0.022\%$ ) and control milk ( $0.72 \pm 0.017\%$ ) were statistically non-significant ( $P > 0.05$ ). SNF content of buffalo milk at MPKP ( $10.46 \pm 0.13\%$ ), was significantly higher ( $P < 0.05$ ) than that of control milk ( $10.06 \pm 0.031\%$ ), but at TAYP ( $10.05 \pm 0.15\%$ ) and CP ( $10.38 \pm 0.12\%$ ), it was relatively similar ( $P > 0.05$ ) to that of control buffalo milk.

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**ISOLATION, PURIFICATION AND PARTIAL CHARACTERIZATION OF LIVER PEROXIDASE ENZYME Pb<sup>+</sup>Cd METAL MIXTURE STRESSED AND CONTROLLED *LABEO ROHITA***

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The purpose of present study was purification and partial characterization of liver peroxidase from Pb+Cd metal mixture stressed and controlled *Labeo rohita*. Purification of liver peroxidase was carried out by the combination of ammonium sulfate precipitations, ion exchange chromatography and Sephadex G-75 superfine gel filtration chromatography. Specific activity of controlled and Pb+Cd metal mixture stressed *L. rohita* was remained 1317.56 and 1289.00 U/mg respectively in gel filtration chromatography. The fold purification of purified liver peroxidase was remained about 16.90 and 22.57 fold for Pb+Cd metal mixture stressed and controlled *L. rohita*. Molecular mass of the purified peroxidase was obtained 65 kDa. with the help of gel filtration chromatography. The purified enzyme was remained active in wide range of pH (4.0-9.0) and temperature (4°C-80°C). Optimum pH and temperature for purified liver peroxidase was obtained 7 and 40°C respectively. The results also indicated that the activity of purified liver peroxidase was remained high in Pb+Cd metal mixture stressed *L. rohita* rather than controlled *L. rohita*.

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**CHARACTERIZATION OF PURIFIED LIVER CATALASE ENZYME FROM CONTROLLED AND COPPER STRESSED *CIRRHINUS MRIGALA***

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Extensive industrialization and urbanization has increased the concentration of

heavy metals into the aquatic environment. The polluted environment of fish may interfere with metabolic activities of fish. Catalase is a well-known antioxidant enzyme and act as a biomarker to protect the fish against the oxidative stress. To studies these interferences freshwater fish, *Cirrhinus mrigala* was exposed to sub-lethal concentration of copper for two weeks. After two weeks exposure period, the controlled and treated fish was dissected and liver was extracted and preserved at -20°C for catalase enzyme purification and characterization. The e catalase enzyme was partially purified by ammonium sulphate precipitation method. Further purification was done by using ion exchange chromatography and gel filtration chromatography. For characterization study, the effect of pH, temperature and substrate concentration was studied. Metal stressed catalase purified by gel filtration chromatography showed specific activity 1516.5 U mg<sup>-1</sup> and controlled liver catalase showed specific activity about 1300 U mg<sup>-1</sup>. Molecular weight of purified catalase was recorded 240 kDa and the molecular weight of its subunits was measured about 58.76 kDa. Optimum pH and temperature was noted 7 and 25°C respectively in both controlled and metal stressed *C. mrigala*. The results further showed that the activity of catalase was observed higher in metal stressed *C. mrigala* liver as compared to controlled *C. mrigala* liver.

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**IMPACT OF CONCENTRATES WITH VARYING LEVEL OF  
METABOLIZEABLE ENERGY AND CRUDE PROTEIN ON GROWTH RATE  
AND NUTRIENT DIGESTIBILITY IN MALE BUFFALO GROWING CALVES**

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Twenty Nili Ravi buffalo male calves of 10 months age and weighing 100± 10 kg were used in a completely randomized design (CRD) to investigate the effect of varying levels of crude protein (CP) and metabolizable energy (ME) on growth rate and nutrient digestibility of male buffalo calves. Four experimental rations A, B, C and D were formulated with two levels of CP *i.e.* 16.0 and 18.0% along with two varying levels of ME *i.e.* 2.82 & 2.94 Mcal/kg. The animals were fed individually ad libitum intakes and all the standard managerial practices were followed. Feed intake (DM basis) kilograms per day and weight gain were higher in buffalo calves fed ration B & D containing 2.94 Mcal/kg, but the difference were non significant statistically among all the groups. However, the results of feed conversion efficiency were better in the animals of group A & C fed on rations containing 2.82 Mcal/kg ME levels. DMI digestibility were significantly higher in the animals fed on diet containing low level of protein other than higher protein level while CP and NDF digestibilities were almost same in all groups and difference were non significant statistically. It was proved that metabolizable energy requirement of Nili Ravi buffalo calves are higher than beef cattle as recommended by NRC (1996).

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**MORUS NIGRA FRUIT PULP EXTRACT CURES CHROMIUM INDUCED HEPATOTOXICITY IN MICE**

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In present study we explored the histopathological and micrometric changes of hexavalent chromium Cr(VI) exposure and their ameliorations upon post treatment of *Morus nigra* (M) fruit pulp extract. Thirty adult male mice were distributed randomly in three groups (10 each) namely control (C), chromium (Cr) and chromium + morus (CrM). C group was maintained without any treatment while Cr and CrM groups were given 50ppm Cr(VI) (from potassium dichromate) in drinking water for 10 days, additionally CrM group was given 0.2ml M fruit pulp extract every 12 hourly from day 11-15. All animals were sacrificed on day16 of the study to obtain liver to obtain mean group hepato-somatic indices and histopathological and micrometric studies. The micrometric data obtained was subjected to appropriate statistical analyses (mean $\pm$ SEM, ANOVA and Duncan's multiple range tests). Results indicate various pathological signs in the hepatic architecture that include misalignments of hepatic cords and necrosis of the hepatocytes leading to fibrosis in Cr treated groups. Interesting signs of recovery seen in CrM group include hepatoblastic proliferations and realignments of hepatic cords. Significantly higher ( $p<0.0001$ ) hepatosomatic index was obtained in Cr (11.54g $\pm$ 1.25) as compared to C (6g $\pm$ 0.33) and CrM (5g $\pm$ 0.13) groups. The micrometric data indicate significant decline in mean number of hepatocytes per unit area, mean cross-sectional area (CSA) of the hepatocytes and the relative area occupied by the hepatocytes; while the mean CSA of hepatocytic nucleus, central hepatic vein, sinusoidal spaces and number of kupffer cells were significantly higher than control in Cr group. Not all but most of these micrometric alterations of Cr exposure were found reversed in CrM group. The results show that five days post treatment of M fruit pulp extract convincingly recovers the histological and micrometric signs of hepatic pathologies of Cr exposure.

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**COMPARISON OF THE CURATIVE POTENTIALS OF VITAMIN E WITH OLIVE AND STRAWBERRY FRUIT PULP EXTRACTS AGAINST FLUORIDE EXPOSURE RELATED HEPATO-HISTOPATHOLOGY IN MICE**

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Hepato-histopathological responses of low dose (50ppm *ad libitum*) fluoride exposure and their ameliorations on post treatment of vitamin E, olive and strawberry fruit pulp extracts were investigated in mice. There were 5 experimental groups of 10 animals each; called as (i) Control (F free drinking water for 15 days); (ii) NaF (F) {50ppm F ion from

NaF in drinking water (10 days) followed by F free water (5 days)); (iii) NaF+vitamin E (FE) {50ppm F ions from NaF (10 days) followed by F free water+60ug Vitamin E using corn oil as vehicle (5 days)); (iv) NaF+ Olive (FO) {50ppm F ions from NaF (10 days) followed by F free water +0.2ml Olive fruit extract (5 days)); (v) NaF+Strawberry (FS) {50ppm F ions from NaF (10 days) followed by F free water+0.2ml Strawberry fruit extract (5 days)); All animals were euthanized on 16<sup>th</sup> day of study to recover liver for hepato-histopathological study. The hepato-histopathologies of fluoride exposure include constricted sinusoidal spaces ( $C=8.62\pm0.21\mu$ ,  $FE=8.19\pm0.23\mu$ ,  $FO=7.76\pm0.28\mu$ ,  $FS=7.02\pm0.23\mu$  and  $F=1.9\pm0.23\mu$ ), enlargement in hepatocytic size ( $F=804.5\pm25.04\mu^2$ ,  $FO=525.7\pm28.12\mu^2$ ,  $FS=498.8\pm23.83\mu^2$ ,  $FE=450.8\pm22.16\mu^2$  and  $C=419\pm16.75\mu^2$ ), declined abundance of kupffer cells ( $FE=7.7\pm0.17$ ,  $C=7\pm0.23$ ,  $FS=6.6\pm0.22$ ,  $FO=6.1\pm0.23$  and  $F=3.97\pm0.18$ ) along with nuclear distortions and various signs of apoptosis. Which were effectively reclaimed best with olive followed by vitamin E and strawberry extract. Based upon these findings we suggest olive fruit extract as the best rescuing agent against hepato-histological changes of environmental chemical (like fluoride) exposure.

#### **PREVENTIVE ROLE OF SOME ANTICARCINOGENS ON THE CHEMICALLY INDUCED CARCINOGENESIS IN BALB C MICE**

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Present study was designed to investigate the hepatoprotective effects of allyl sulfide (5 mg kg<sup>-1</sup> b. w.) and vitamin A + carbon tetrachloride (1 mg + 0.1 ml kg<sup>-1</sup> in olive oil) against lead nitrate (8 mg kg<sup>-1</sup> b. w.) and nitrobenzene (100 mg kg<sup>-1</sup> b. w.) induced carcinogenic changes in Balb C mice. The study included the analysis of activities of enzymes ALAT, ASAT and LDH in plasma and liver and some biochemical components like total protein, soluble protein, and cholesterol, DNA and RNA, in liver. The biochemical observations were supplemented with histopathological examination of liver sections. Lead nitrate treatment resulted in significant decrease in LDH activities of plasma and liver and ALAT and ASAT activities increased in liver but remain unaltered in plasma. Among biochemical components soluble protein and RNA were significantly increased but the activity of total protein, cholesterol and DNA remained unaltered by lead treatment. Administration of nitrobenzene resulted in significant decrease in level of LDH in both liver and plasma while ALAT and ASAT activities were significantly decreased in liver but remained unaltered in plasma. Among the biochemical components total protein was significantly decreased but soluble protein, DNA and RNA remained unaltered by nitrobenzene treatment. Allyl sulfide post-treatment markedly prevented the lead nitrate induced decrease in the level of LDH in both plasma and liver. Allyl sulfide failed to abolish lead nitrate induced increase in ALAT and ASAT activities

in liver but significantly decreased the ALAT and ASAT activities in plasma. Among the biochemical components allyl sulfide significantly prevented lead nitrate induced increase in soluble protein and RNA in liver. Allyl sulfide also significantly reduced the total protein in liver. Post-treatment of vitamin A + Carbon tetrachloride failed to abolish nitrobenzene induced decrease in LDH activities in plasma and liver. Among the biochemical components, vitamin A + carbon tetrachloride resulted in significant increase in cholesterol and RNA, while a significant decrease in hepatic DNA. Histopathological observations of the liver sections supported the hepatoprotection by anticarcinogens.

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#### **ESTIMATION OF IRON IN LACTATING AND NON-LACTATING BUFFALOES IN DISTRICT GUJRAT**

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The present study was conducted to determine the serum iron concentration in lactating Nili-Ravi and non-lactating buffaloes (*Bubalus bubalis*) in Tehsil and District Gujrat during the year 2013. A total of four dairy farms in Tehsil Gujrat were selected for this study and 40 blood (10 ml) samples from jugular vein of each were collected from lactating and non- lactating buffaloes. Iron concentration was estimated with the help of Spectrophotometer. There was significant difference ( $P<0.05$ ) between serum iron levels of lactating and non- lactating buffaloes. Trace mineral deficiency causes harmful effect to the growth and reproduction rate. Nutritional deficiencies can be treated through dietary supplementation.

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#### **COMPARISON OF GROWTH PERFORMANCE, PROXIMATE COMPOSITION AND FATTY ACID PROFILE OF TWO MAJOR INDIAN CARPS UNDER THE INFLUENCE OF UREA AND FEED SUPPLEMENTATION**

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The correlation of proximate composition and fatty acid content of the meat of warm water fishes Rohu (*Labeo rohita*) and Mori (*Cirrhinus mrigala*) were determined in relation to body weight. In proximate analysis, *C. mrigala* had maximum value for crude protein and carbohydrate. There was inverse relation between moisture contents and crude lipids. In the analysis of fatty acid profile of carps, PUFA composition of *L. rohita*

and *C. mrigala* was 75.56% and 67.53%, respectively. Among the SFAs the most abundant fatty acids were the palmitic acid (16:0) and stearic acid (18:0) and mono-unsaturated fatty acid palmitoleic and oleic acids were dominant in *L. rohita*. In present project, the maximum value of mono unsaturated fatty acid was observed for *C. mrigala* (76.59%). Here palmitoleic and oleic acids were the dominating mono-unsaturated fatty acids in *L. rohita*. The ratio of PUFA/SFA for above mentioned fish species was 1.7% and 1.4% respectively. This ratio was maximum for *Labeo rohita*.

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#### PREVALENCE OF MALNUTRITION IN CHILDREN AGED <5 YEAR IN DISTRICT SANGHAR, PAKISTAN

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Increasing evidence suggests the majority of children around the world suffer from malnutrition. High mortality rate in children is associated with malnutrition. After the age of six month, the children have to take the weaning diet, which can fulfill the nutritional requirements of the children. However, in the poor community, the diet of children has not met the criteria of WHO recommendation for diet. Malnutrition can be considered a wide spread phenomenon in Pakistan. Poverty and illiteracy has been reported as the major factors contributing to malnutrition children under five. The condition worsens when the area is affected by floods or other emergency conditions. Various anthropometric indicators are used to assess the nutritional status in children. However, measurement of Middle upper arm circumference (MUAC) has been adopted an easy, quick and precise method to detect malnutrition in children under five year age group in emergency situations. The study was conducted in Sanghar District. This cross-sectional study was carried out on 511 children. The children were aged between 6 months to 59 months. The data of their socio-economical condition and the status of malnutrition were collected by interview through structured questionnaire. Malnutrition was described by measuring the middle of the Upper Arm Circumference. Out of 511 children, 338 (66.1%) were affected by malnutrition, further analysis into the mild ( $\leq 13.5$  cm<sup>2</sup>), moderate (11.5-12.5 cm<sup>2</sup>) and severe malnutrition ( $< 11.5$  cm<sup>2</sup>) was carried out. This analysis shows that 221 (43.2%) of the children had mild malnutrition, 65 (12.7%) suffered from moderate malnutrition and 52 (10.2%) had severe malnutrition. Severe malnutrition was significantly higher ( $P < .05$ ) in female children than male children. Malnutrition was significantly higher in older children aged 24-59 months ( $P < .05$ ) than in younger children 6-23 months. This study gives an indication that female children are severely malnourished and malnourishment in older children increases with age.

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**MOLECULAR WEIGHT AND POTENCY COMPOSITION OF VENOM  
PRODUCED BY SCORPION SPECIES (*ANDROCTONUS AUSTRALLIUS* AND  
*HETEROMETRUS INDUS*)**

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The research was conducted to study the molecular weight and potency composition of venom produced by two scorpions species (*Androctonus australis* and *Heterometrus indus*) during April – October, 2013. The venom was obtained from two scorpion species *Androctonus australis* and *Heterometrus indus* and were mixed with PBS (Phosphate buffer solution). After vortex and centrifuge (14,000 rpm for 20 minutes). The supernatant was kept frozen until use. By the SDS-PAGE analysis of these two species, it was found that band numbers 10, 26, 30, 40, 42, 55 and 200 kDa were found in *Androctonus australis* only. Band numbers 14, 15, 25, 60, 90, 95, 100 and 130 kDa were found in *Heterometrus indus* only. While, both species venom have following bands in common 35, 45, 70, 85, 110, 120, 140 and 150 kDa. Hence it has been found that *Heterometrus indus* has more number of protein and more bands with low molecular weight than *Androctonus australis*. The results revealed that 2mg/kg venom was LD<sub>50</sub> of *Androctonus australis* while 2mg/kg venom of *Heterometrus indus* was not.

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**EFFECT OF INDUSTRIAL POLLUTION ON HEAVY METAL UPTAKE,  
OXIDANT AND ANTIOXIDANT STATUS AND HISTOLOGY OF LIVER OF  
*CATLA CATLA* INHABITING THE RIVER CHENAB**

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Food safety is a major public concern worldwide. The increasing demands for food and food safety has drawn the attention of researchers to the risks associated with consumption of contaminated foodstuffs *i.e.* pesticides, heavy metals and or toxins in fish and other organisms. Heavy metals contamination is a major problem of our environment and they are also one of the major contaminating agents of our food supply. This problem is receiving more and more attention all over the world, in general and in developing countries in particular. Keeping in view above facts this study was designed to assess the effects of heavy metals (Pb, Zn, Cd, Ni, and Cr) on oxidant, antioxidant and histology of liver of *Catla catla*. The fish samples were collected from one control site (Kote Khera) which was relatively clean and three polluted sites viz., Thatha Muhammad Shah (heavily polluted), Patan Draj (medium polluted) and Head Trimue (less polluted) of River Chenab. Fish samples were collected fortnightly for three months and were analysed for



the heavy metals (Pb, Zn, Cd, Ni, and Cr), oxidant and antioxidant status and histology of liver. The concentrations of all selected heavy metals in fish liver collected from three polluted sites were higher than the international recommended limits for food fish. Whereas, metal levels in fish collected from reference site were within the permissible limits. Hence, these could be a major health risk for fish and consumers. In this study higher oxidation status was investigated in fish sampled from Thatha and antioxidant status was found to be higher in control site (Kote Khera) as compared to fish sampled from Trimue and Draj. The pronounced histological alternations were observed in the liver of *Catla catla* collected from the polluted sites. The sever alternation was observed in the fish sampled from Thatha station due to the presence of the heavy load of these metals as compared to the control site. It can be concluded that oxidant, antioxidant and histological evaluation is a useful and reliable biomarkers for revealing environmental contamination.

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**PESTICIDE ANALYSIS IN *CYPRINUS CARPIO*, WATER AND SEDIMENTS  
SAMPLED FROM THE RIVER INDUS IN MIANWALI DISTRICT**

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Aquatic environmental pollution is a very serious and on growing problem. Various deleterious effects on aquatic organisms occur when agricultural, industrial and commercial chemicals discharge into the aquatic environment. Aquatic organisms in which fish are also included accumulate these pollutions directly from the polluted water and indirectly from the food chain. This study analysed different types of pesticides in *Cyprinus carpio*, water and sediments sampled from the River Indus around Mianwali District. This study involved three sites around the River Indus in Mianwali District. Kukranwala (S1) site was designated as the reference site or low polluted site because of relatively less human activities in its surrounding areas. The other two sites called Rokhri (S2) and Ballo Khel (S3) were designated as medium and heavy polluted sites, respectively. Selected pesticides namely Imidacloprid, Acetameprid, Thiadocloprid, Thiamethoxam, 6-chloronicotinic acid, Carbofuran, Cypermethrin, Lemda-cyhalothrin and Delta-methrin were analyzed in the fish muscles, river sediments and water by high performance liquid chromatography (HPLC). Nine standards were run but only Delta-methrin residues were determined in fish species and sediment samples. Deltamethrin in *Cyprinus carpio* ranged from 0.490-0.839 µg/g, which exceeded FAO-WHO maximum residual limits (0.5mg/Kg) and in soil it ranged from 0.214-0.318. No pesticide residues were detected in the water sampled from all sites. This study revealed that fish from river Indus is safe for human consumption with reference to different pesticide residues except delta methrin.

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**FRACTIONATION: A USEFUL TECHNIQUE TO ENHANCE THE NUTRITIONAL AND FUNCTIONAL CHARACTERISTICS OF MILK FAT**

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Milk fat is profoundly condemned for possessing the maximum concentration of atherogenic fatty acids amongst all the dietary lipids. The connection of Myristic, Lauric and Palmitic acids with hypercholesterolemia is scientifically proven. The correlation between extent of saturated fatty acids of milk and dairy products with cardiovascular diseases has also been well established. The increased knowledge of health related disparities due to the intake of milk and dairy products has led to a decline in their consumption; about 44% of the American population has shown their concerns regarding the consumption of milk as a regular part of their diet. The British, European and American Heart Associations recommend lowering the intake of saturated fatty acids to minimize the risk of cardiovascular diseases. The use of milk fat is restricted to butter, butter oil and anhydrous milk fat as compared to hydrogenated fats which offers a high degree of suitability in a wide range of baked goods and large number of food preparations. Fractionation of vegetable fats and oils is a common, industrially performed practice to modify the physical, chemical and functional characteristics of plant based fats and oils with the generation of harmful *trans* fatty acids. However, little is known regarding the enhancement of nutritional and functional characteristics of through the fraction process of fat modification. The research work has shown that the concentration of saturated and hypercholesterolemic fatty acids can be considerably decreased through the fractionation technique. Milk fat is exceptional among all the dietary lipids for possessing the short chain fatty acids, which confer a typical taste and aroma to milk based stuffs, their loss or drop in the modified version of fats could have a great impact on the sensory attributes. Among all the fat modification strategies employed for the modification of fats, fractionation is a unique process that enhances the concentration of short-chain fatty acids in the modified version of milk fat. Olein fraction of milk fat can be successfully used in the formulation of functional ice cream, and butter with reasonable oxidative stability and acceptable sensory characteristics. The problem of lack of functionality in milk fat can be resolved by fractionating the milk fat various temperatures and stearins can be successfully used as plastic fats and superior shortenings.

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### PHYSICO-CHEMICAL CHARACTERISTICS AND OXIDATIVE STABILITY OF OLEIN BUTTERS

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Physico-chemical characteristics and oxidative stability of olein based butters were investigated. Cream (40% fat) was fractionated into olein and stearin fractions at 25, 15 and 10°C, olein fractions were turned into butter, designated as 0-25, 0-15 and 0-10, respectively, compared with a normal butter, stored at -10±1°C for 6-months. The conversion of oleins into butter did not reveal any issue of standard of identity. The concentration of C18:1 and C18:2 dropped to 12.3% and 48% in six months stored 0-10. Peroxide value and conjugated dienes of 0-10 increased from 0.18 to 3.59 (meqO<sub>2</sub>/kg) and 0.09 to 3.53 as compared to control; 0.18 to 1.26 (meqO<sub>2</sub>/kg) and 0.09 to 1.54, after 180-days of storage. Induction period of control was higher ( $p < 0.05$ ) than the fresh and stored olein butters. Taste, smell and overall acceptability of the olein butters were not different from the control till 60-days of storage, after that, sensory score started to deteriorate. Texture of all the treatments was softer than the control; butter can be manufactured from low melting fractions of milk fat with increased health benefits, acceptable sensory characteristics but should be consumed within two months from the date of manufacturing.

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### BETA CYCLODEXTRIN: AN EFFICIENT AND SAFE ADSORBENT FOR THE REMOVAL OF CHOLESTEROL FROM BOVINE MILKS

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The role of dietary cholesterol in the enhancement of potentially hazardous low density lipo-proteins has been well established. In recent years, the role of beta cyclodextrin for the removal of cholesterol from milk and its products has been extensively studied. Beta cyclodextrin is composed of seven glucose units linked each other in the form of a ring, the size of inner cavity of this oligosaccharide is exactly

similar to the size of cholesterol molecule, the adsorption of cholesterol is further enhanced by the lipophilic characteristic of the inner side of the ring. The outer side of the ring has hydrophobic nature, which offers a unique benefit of cholesterol removal from bovine milks without being dissolved in the aqueous phase. Beta cyclodextrin has been widely used for the removal of odours from foods for over 2-decades in Japan, generally recognized as safe, fully metabolized by the intestinal micro flora. Of the several techniques, the cholesterol removal from bovine milks was most efficiently achieved by beta cyclodextrin. The spent beta cyclodextrin can be re-activated by cross-linking with adipic acid, which makes the cholesterol removal process more efficient with minimum generation of industrial waste. It can remove up to 90% cholesterol at lower concentrations without affecting the sensory characteristic of pasteurized milk, cream cheese, butter, yoghurt and cheese. Beta cyclodextrin can be used for the preparation of functional dairy products with lower contents of cholesterol with minimum loss of vital bio-active compounds of bovine milk.

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**STABILIZATION OF BUTTER AT AMBIENT TEMPERATURE THROUGH  
DATE PALM (*PHOENIX DACTYLIFERA* L.) FRUIT EXTRACT DURING  
WINTER**

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Oxidative stabilization of butter at ambient temperature was achieved in winter through ethanolic date palm (*Phoenix dactylifera* L.) extract. Date extract (DE) was characterized for total phenolics, DPPH free radical scavenging activity and inhibition of nitric oxide macromolecules. Butter was added with 200, 400 and 600-ppm DE (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively) compared with a control, stored at ambient and refrigeration temperature for 90-days (November, December and January). The effect of DE on oxidative stability and sensory characteristics of butters at ambient and refrigeration temperatures were investigated. The oxidative stability of butters stored at refrigeration temperature was superior to the butter stored at ambient temperature; however, the butter supplemented with 600-ppm date extract yielded the lowest extents of primary and secondary oxidation products, whereas the un-supplemented butter underwent serious oxidative consequences. The sensory characteristics of supplemented butters were not different from the control (P>0.05). The results indicate that ethanolic date palm extract at 600-ppm concentration can be used for the long term storage of butter at ambient temperature during winter months.

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**EFFECT OF *NIGELLA SATIVA* OIL ON LIVER AND KIDNEY FUNCTION OF BROILER CHICKS FED ON HIGH FAT DIET**

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This study was conducted to determine the effect of *Nigella sativa* oil on hepatic and renal functions of broiler chicks fed on high fat diet of animal origin and high fat of vegetable origin. The chicks were divided into six groups. Group 1 was fed on CPF, Group 2 was fed on CPF and *Nigella sativa* oil @ one drop per 50 gram body weight, Group 3 was fed on high fat (30% vegetable ghee) diet only, Group 4 was fed on high fat (30% vegetable ghee) *Nigella sativa* oil @ one drop per 50 gram body weight, Group 5 was fed on high fat (30% beef tallow) only and Group 6 was fed on high fat (30% beef tallow) diet and *Nigella sativa* oil @ one drop per 50 gram body weight. The conc. of different enzymes present in the serum like Bilirubin, Creatinine, GOT, GPT and Urease decreases significantly during six weeks of post hatching life in *Nigella sativa* oil administered chicks.

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**CHANGES IN THE ANTIBODY TITER OF *LABEO ROHITA* FOLLOWING BATH OF LEVAMISOLE**

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In this study, the immunostimulatory potential of levamisole was examined by giving a bath of levamisole for a period of five weeks to 30 healthy specimens of *Labeo rohita* with average body weight of  $230.21 \pm 50.16$ . Throughout the experimental period fish were fed with natural feed present in the pond. Fish were divided into five groups randomly, first group served as control with application of levamisole at the rate of 0 mg/L while other four groups were given levamisole treatment at the rates of 2.5 mg/L/ 30 minutes, 2.5 mg/L/ 60 minutes, 5.0 mg/L/ 30 minutes, 5.0 mg/L/ 60 minutes, respectively. Blood samples were collected at intervals of one week for serum separation. One week post treatment with levamisole fish specimens in experimental groups were injected with sheep red blood cells and then changes in the antibody titer of *Labeo rohita* at 7<sup>th</sup> and 14<sup>th</sup> day post injection with Sheep RBC's (SRBC) were recorded by means of microplate hemeagglutination test. The geometric means of antibody titers showed that Total anti SRBC antibody titer, Ig M anti SRBC antibody titer and Ig M anti SRBC antibody titer were significantly higher in serum of levamisole treated fish as compared to control. Moreover highest antibody titer was recorded in group treated with levamisole at the rate of 5.0 mg/L/ 60 minutes providing an evidence of concentration and time related effect of the immunostimulant under study.

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**GENETIC DIFFERENTIATION REVEALED BY ISOZYME VARIATIONS  
AMONG THE CRABS OF FAMILY OCYPODIDAE FOUND ALONG THE  
COAST OF PAKISTAN**

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It has been generally observed that isozyme variation is considered as a possible marker of genetic differentiation among the populations. Ocypodid crabs were collected from three different Populations sites of Pakistan (Sandspit, Sonari and Sonmiani). Polyacrylamid vertical slab-gel electrophoresis was used to investigate the genetic variations in two enzymes and one general protein among four species (*Uca iranica*, *U. sindensis*, *Ocypode rotundata* and *O. ceratophthalma*) of the family Ocypodidae. A total 18 polymorphic loci were investigated in four species of crabs. The relationship of isozyme variations within the different species of ocypodid crabs were determined isozyme loci (CD1, CD2, & CAT1, CAT2) and general protein loci (GP1, GP2, GP3,). Electrophoresis of two enzyme loci and one general protein revealed that moderate levels of genetic differentiation which showed the mean percentage of Polymorphic loci 62% & 71% among genus *Uca* and *Ocypode* respectively. The mean observed heterozygosity were shown by polymorphic loci was 0.31 among the populations of genus *Uca* when compared to the genus *Ocypode* populations which showed the mean observed heterozygosity was 0.43. Mean allelic frequencies observed for the two isozyme loci (CD1 0.15 and CD2 0.18, CAT1 0.090 and CAT2 0.91) and for general protein (GP1 0.151, GP2 0.278, and GP3 0.571) in genus *Uca* and (CD1 0.402 and CD2 0.598, CAT1 0.26 and CAT2 0.74) and one general protein (GP1 0.127, GP2 0.21, and GP3 0.66) in genus *Ocypode*.

**PURIFICATION AND PARTIAL CHARACTERIZATION OF LIVER  
CATALASE ENZYME FROM CONTROLLED AND Pb+Cd METAL MIXTURE  
STRESSED GRASS CARP (*CTENOPHARYNGODON IDELLA*)**

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Enzymes are necessary for normal cellular metabolism. Catalase enzymes play an important role in the protection of tissues from the toxic effects of reactive oxygen species. The present study was designed to partially characterize liver catalase enzyme

after purification from controlled and metal mixture stressed *Ctenopharyngodon idella*. Purification steps were included partial purification with the help of ammonium sulphate precipitation, ion exchange chromatography by using DEAE-Cellulose and gel filtration chromatography by using Sephadex G-150 resins. In gel filtration chromatography, maximum specific activity for metal mixture stressed and controlled *C. idella* was noted 851.85 and 1447.40 UmgL<sup>-1</sup> with fold purification of 8.39 and 7.21 respectively. Optimum pH at which purified liver catalase enzyme showed maximum activity was noted 7.0 for both metal mixture stressed and controlled fish *C. idella* and the temperature at which enzyme showed maximum activity was noted 25°C. The K<sub>m</sub> value of controlled *C. idella* purified liver catalase was noted 6 mM H<sub>2</sub>O<sub>2</sub> while for the stressed *C. idella* it was noted 7.66 mM H<sub>2</sub>O<sub>2</sub>. Inferences of present study further showed that catalase enzyme activity was high in controlled *C. idella* as compared to stressed *C. idella* where the metal mixture exposure inhibited the normal enzyme activity.

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#### GENETIC VARIATION AMONG THE WILD AND HATCHERY RAISED POPULATIONS OF *LABEO ROHITA* REVEALED BY RAPD MARKERS

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The studies on genetic diversity of *Labeo rohita* by using molecular markers were carried out to investigate the genetic structure by RAPD marker and the levels of polymorphism and similarity amongst the different groups of five populations of wild and farmed types. The samples were collected from different five locations as representatives of wild and hatchery raised populations. RAPD data for Jaccard's coefficient by following the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for Hierarchical Clustering of the similar groups on the basis of similarity amongst the genotypes and the dendrogram generated divided the randomly selected individuals of the five populations into three classes/clusters. The variance decomposition for the optimal classification values remained as, 52.11% for within class variation while 47.89% for the between class differences. The Principal Component Analysis (PCA) for grouping of the different genotypes from the different environmental conditions was done by Spearman Varimax rotation method for bi-plot generation of the co-occurrence of the same genotypes with similar genetic properties and specificity of different primers indicated clearly that the increase in the number of factors or components was correlated with the decrease in eigenvalues. The Kaiser Criterion based upon the given values greater than one, first two main factors accounted for 58.177% of cumulative variability.

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### THE EFFECT OF TEMPERATURE AND CUTTING TIME ON CURD YIELD AND QUALITY MADE FROM BUFFALO AND COW MILK

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For the present study, two species of lactating animals *i.e.* Bubalus bubalis (Buffalo) and Bos indicus (Cow) was selected. In both types of milk, highest curd yield at 34°C was obtained at 60 minutes of cutting time after the addition of rennet followed by 39°C at 60 minutes of cutting time while at 28°C and 60 minutes of cutting time the yield was low and cutting times. This may be due to the improper rearrangements of casein network and the minimum curd fat retention level. The % total solids of curd were maximum at 28°C and at 60 minutes of cutting time may be due to the lower fat losses in whey and %TS were lower at 34°C when the cutting time was 90 minutes after the rennet addition because of the higher moisture content retention in the casein network due to the elongation in the protein network rearrangement time. The whey fat losses and curd fat retention values were minimized and maximized, respectively, between 28 and 39°C. The whey fat losses (WFL) and curd fat retention (CFR) were minimum at 28°C and maximum at 39°C. Increasing cutting time at 28°C results in firm gel with a good capability of fat retention and will decrease fat losses in whey. However, at temperature more than 30°C, coarsening of the milk gel occurs more rapidly, permeability of the gel is greater and the microsyreresis can occur at longer aging times. All these factors decreased the ability of the curd to retain fat. These results showed that the retention of fat is dependent on relative rigidity and structure of network at cutting. Below 28°C, there will be less gel firmness before cutting, therefore, increase in temperature will increase the rate of curd firming at best gel will be formed at 35°C. Above 35°C, the network becomes more rigid, rapid coarsening occur and the gel is more porous and all these assists the release of fat in whey and also the fat has high mobility at higher temperature which results in increase fat losses.

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### REDUCTION OF TELLURITE BY GLUTATHIONYL-HYDROQUINONE REDUCTASES

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Tellurium (Te) and its compounds are widely used in petroleum refining, the electronic and photoelectronic industries, optics, glass, and sensors. However the recent expanded use of Te leads to the environmental contamination. In the environment,



soluble oxyanions tellurite (TeO<sub>3</sub><sup>2-</sup>, Te (IV)) is highly toxic to both eukaryotic and prokaryotic cells at a concentration as low as 1 µg/ml. Microbial processes can contribute to Te(IV) reduction by direct enzymatic reduction, or through indirect redox active organic molecules, such as quinines. Five recombinant proteins after purification were used to reduce Te. Kinetic analysis was also performed. Reduction of Te with suitable time intervals by *E. coli* K-12, mutant and complementary strains were also performed. Plate survival assays of the above mentioned strains were also done.

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#### **CITRIC ACID PRODUCTION FROM *ASPERGILLUS NIGER* USING MANGO PEEL**

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Citric acid is an important organic acid which is being globally consumed and produced in large numbers. In the present study, mango peel was utilized for citric acid production by *Aspergillus niger* and the fermentation parameters were optimized. Maximum yield of citric acid was obtained using mango peel 11%, sucrose concentration 5%, inoculum 2%, potassium dihydrogen phosphate and ammonium nitrate at pH 5 and 32° C after 8 days of fermentation.

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#### **RAPD MARKER TECHNIQUE, A TOOL FOR CHARACTERIZATION OF NATIVE HONEY BEE SPECIES POPULATIONS**

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The current study was planned to estimate the genetic variations among two different populations from district Nankana and Narowal, Punjab, Pakistan. Samples of worker bees of *Apis dorsata* were collected and preserved in 50% alcohol in plastic jars. The wings and legs of the bees were homogenised in pestle and mortar to extract the DNA. It was amplified by using RAPD (Random Amplified Polymorphic DNA) marker technique to find out genetic differences among the honeybee populations on the basis of their distribution in these districts. Two different primers (Moh-13 and OPA-07) were used. The OPA-07 was expressed more genes than Moh-13. The findings would be helpful to make a good programme to get better health of honey bees.

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### **A QUANTITATIVE HEMATOCRIT ANALYSIS OF INDUCED HEAVY METALS IN BIRDS**

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A study was conducted to investigate the effects of feeding inorganic heavy metals such as Zinc, Copper, Arsenic and lead on the blood chemistry of domesticated birds. The 25 pigeons were divided in 5 groups contained one group as a control. The number of red blood cells significantly decreased, while white blood cells increased. The affected birds showed low packed cell volume (pcv) as compared to the control group. Total serum protein and albumin with simultaneous reduction was also observed. The significant value of alanine amino-transferase and cholesterol was also observed in two weeks. The urea, uric acid and creatinine were at higher level probably due to renal failure. Among heavy metals Cu and Zn showed less diverse effects as compared to Pb and Ar. The arsenic and lead showed drastic effect on domesticated birds. A significant reduction in hemoglobin concentration, hematocrit and red blood cell count was also observed.

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### **THE HAEMATO-PHARMACOKINETICS OF ANTI-INFLAMMATORY DRUGS AND ITS EFFECTS ON SERUM BIOCHEMISTRY OF *COTURNIX COTURNIX***

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Haematology and serum biochemistry study may provide valuable information about the physical condition of individuals, making them a useful tool to differentiate healthy animals from affected animals. In the present study, we observe haematological parameters (RBCs & WBCs count, PCV%, MCV and blood pH) and clinical biochemistry (albumin, total protein, urea, uric acid, cholesterol, creatinine, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and CK-NAC) level in serum. We aimed to ascertain NSAIDs safety levels in birds through *ex-situ* studies at normal therapeutic doses. A total of 60 common quails were divided in 6 groups; one control and 5 treated groups. Every group was further divided in two sub groups; 1<sup>st</sup> group for 10 days long trial and 2<sup>nd</sup> group of 20 days. NSAIDs were given at weight dependent therapeutic dose of meloxicam 1.8 mg/kg, mefenamic acid 2mg/kg, ibuprofen 2mg/kg, diclofenac potassium 2mg/kg and paracetamol 2mg/kg, 3x a day. The clinical signs observed as depression, somnolence, increased body temperature, severe swelling on feet, droopiness, diarrhoea and lethargy in all treated groups except control. However partial mortality occurred in mefenamic acid, meloxicam and diclofenac potassium treated groups. Analysis of data reveals that, NSAIDs had acute effects on birds'

(*Coturnix coturnix*) haematological parameters. The haematological effects of mefenamic acid showed an increase in RBCs count and WBCs count in all trials. On the other hand, all other NSAIDs' showed decrease in RBCs & WBCs count, PCV% and MCV. Effect of NSAIDs on serum biochemistry profile, indicated severe damage to vital organs, especially, to liver and kidney indicating an increase in ASAT, cholesterol, total protein, albumin urea and uric acid level. However, some parameters including CK, creatinine and ALAT levels were not affected.

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## 2. CELL BIOLOGY

### EVODIAMINE SENSITIZES U87 GLIOBLASTOMA CELLS TO TRAIL VIA DEATH RECEPTOR PATHWAY

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Tumor necrosis factor  $\alpha$  (TNF-  $\alpha$ )-related apoptosis-inducing ligand (TRAIL) has been shown to selectively kill cancer cells without affecting normal cells. Most glioma cells are resistant to TRAIL-induced apoptosis. Resistance to TRAIL limits its potential as a drug for glioma therapy. The present study was conducted to identify bioactive compounds that have potential to sensitize U87 glioblastoma cells to TRAIL. Evodiamine a major bioactive compound of Chinese herb *Evodiae fructus* was found to sensitize U87 glioblastoma cells to TRAIL. TRAIL and evodiamine, in combination or alone were used to treat U87 glioblastoma cells. We found that evodiamine inhibited the growth of cells in a dose-dependent manner; however, TRAIL alone failed to exert any cytotoxic effect. Combining TRAIL with evodiamine significantly increased the apoptosis rate in U87 glioblastoma cells as compared to evodiamine alone. Further mechanistic study showed that evodiamine/TRAIL synergistic effect was associated with up-regulation of death receptor 4 (DR4), death receptor 5 (DR5) cleaved caspase-8 and cleaved caspase-3. The present study demonstrated for the first time that evodiamine can sensitize U87 glioblastoma cells to TRAIL via death receptor pathway. Thus our results suggest that co-treatment with evodiamine and TRAIL could represent a novel chemotherapeutic strategy for glioma therapy.

### ARTERIAL VASCULAR CELL LINE EXPRESSING SSAO: A NEW TOOL TO STUDY THE PATHOPHYSIOLOGY OF VASCULAR AMINE OXIDASES

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Semicarbazide-sensitive amine oxidases (SSAO) widely exist in nature, mainly expressed at significant levels in vasculature. It plays a detrimental role in vascular diseases, particularly atherosclerosis, which occurs mainly in arteries. Herein we for the first time present SSAO expression in arterial lineage of vascular cell line *i.e.* human

umbilical arterial endothelial cell (HUAEC). Firstly, two commercially available gene transfection reagents were compared to determine high transfection efficiency and then the expression behavior of HUAEC:SSAO was characterized. Furthermore, our model was also been compared with commonly used human embryonic kidney (HEK) cell transfected with the same vector. For enzymatic assay, an in-house developed highly sensitive high performance liquid chromatography electron spray ionization mass spectrometry (HPLC-ESI-MS/MS) method was applied. Results indicated that the maximal transfection efficiency in HUAEC was detected by JetPEI™ and transfected protein was expressed at membrane and cytosol of different clones. No significant variations were observed in HUAEC between cell passages 1 to 7, although HEK cell displayed 2 fold higher SSAO expression level than HUAEC. The transfected SSAO was shown to be released into the cell-culture medium. Both cellular and released types of SSAO exhibited monomer and dimer structural forms. The cytotoxicity determination exhibited large number of viable cells after transfection with JetPEI™. Differential expression characterization of this new cell line demonstrates the correct behavior of SSAO in arterial endothelial cells and also provides a real physiological environment to elucidate the unclear role of this enzyme. In addition, our cellular model could partly solve the problems raised by the loss of enzyme expression found in cultured endothelial cells. This model could also be a useful tool for proteomic base study, screening of interacting protein and analysis of compounds that could modify its activity for therapeutic purposes.

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#### **EFFECT OF GALLIC ACID IN REDUCING CYTOTOXICITY OF THERMALLY OXIDIZED TALLOW**

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Gallic acid (3, 4, 5-trihydroxybenzoic acid) is one of the naturally occurring plant polyphenol that is richly found in tea, apple peels, red wines, sumac, grapes, gallnuts, oak bark and other edible plants. Gallic acid (GA) possesses various biological properties, including antioxidant, anticancer and anti-inflammatory activities. Phenolic hydroxyl (OH) groups in the GA structure are responsible for its ability to suppress oxidative stress by exhibiting free radical scavenging activities and products of lipid oxidation, which are easily absorbed by animals and may induce oxidative stress via free radicals chain reaction. This diminishes cell survival because they impair membrane related functions through oxidative damage of lipids and protein modification. Tallow is a form of animal fat obtained from the rendered animal bones and soft tissues consisting mainly of triglycerides. In Pakistan, tallow is used for the frying of traditional chapli and shami kebab. During frying, the beef tallow is exposed continuously to uncontrolled high temperature, air and moisturizer is oxidized. The oxidized tallow enters the food matrix and produces toxic effect on human health by increasing reactive oxygen species (ROS),

LDL-cholesterol and enhances the risk of coronary heart diseases and cancer. The aim of the present study was to investigate the potential of GA on hematological, biochemical and histopathological parameters to alleviate the cytotoxicity of thermally oxidized tallow in rabbits. Tallow was obtained from market and thermally oxidized on hot plate at  $140 \pm 10^{\circ}\text{C}$  for 18 hrs. Sixty rabbits of local Himalayan strain were selected as experimental model and divided into ten groups. Thermally oxidized tallow and gallic acid were administered orally per kg body weight for two weeks as follows: Group A (control), Group B (3g/kg.b.w thermally oxidized tallow, OT), Group C (3g OT+20 mg GA), Group D (OT +40 mg GA), Group E (3g OT+60 mg GA), Group F (3g OT+80 mg GA), Group G (20 mg GA), Group H (40 mg GA), Group I (60 mg GA), Group J (80 mg GA). Blood samples were collected from jugular vein at 7<sup>th</sup> day and 14<sup>th</sup> day of the experiment for hematological assessment and estimation of serum triglycerides and total cholesterol concentration. Rabbits were then sacrificed and liver and kidney tissues were taken for histopathological findings. With regard to hematological parameters: WBC, RBC, Hb, HCT, MCV, MCH and MCHC, no significant difference was found in any of the treatment groups when compared with the control or with each other. Thermal oxidation of tallow led to an increase in serum triglycerides (TG) and total cholesterol (TC) concentrations. Gallic acid supplementation (60 and 80 mg) with thermally oxidized tallow significantly lowered serum TG as compared to oxidized tallow group. Rabbits treated with 20 mg GA alone also showed a significant decrease in TG concentration as compared to OT group. Similarly, dose dependant GA supplementation (40, 60 and 80 mg) with oxidized tallow significantly decreased serum TC concentration as compared to the OT group, whereas high doses of GA alone increased serum TC concentration. Oxidative stress caused by oxidized tallow also produced degenerative changes in liver and kidney tissues. However, a degenerative effect of thermally oxidized tallow was reduced upon co-treatment of different doses of GA. These results suggested that GA treatment reduced cytotoxicity induced by oxidized tallow (lipids) and indicated the protective effect against oxidative stress raising the possibility of gallic acid to be used a health supplement.

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#### ***IN VITRO* ANTIOXIDANT EFFECT OF *CAMELLIA SINENSIS* ON HUMAN CELL CULTURES**

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Free radicals put in to more than one hundred disorders in humans. Environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress contribute in their production. They cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Recently there has

been an upsurge of attention in the therapeutic potentials of medicinal plants. Numerous natural antioxidants have already been isolated from different varieties of plant material such as leaf vegetables, fruits, seeds, cereals and algae. Their protective mechanism can be explained by the capacity of the antioxidants compounds *e.g.* phenolics, flavonoids and polypropanoids in the plants and plant products themselves to scavenge free radicals, due to their proton donating ability. Research methodology of present study involved isolation and extraction of plant extracts, screening of phytochemical components, determination of antioxidant activity and its effect on living cells using lymphocytes as a model system. *Camellia sinensis* (green tea) extracts were prepared in three different solvents viz. aqua, methanol and ethanol. Initially, phytochemical screening involved determination of ascorbic acid, phenolic, flavonoids and flavonol contents. Antioxidant activity determination included scavenging assays *e.g.* DPPH assay and ABTS assay, HPLC, hydrogen peroxide scavenging assay and reducing power assay. *In-vitro* antioxidant actions were determined by the activities of catalase (ELISA kit method), superoxide dismutase, lipid peroxidation and total protein contents on lymphocyte cell lines. The present study revealed that *Camellia sinensis* has high contents of ascorbic acid, phenols, flavonoids, and flavonol. It was also a good scavenger of oxidants as evident by DPPH, ABTS and reducing power assay. *In-vitro* antioxidant results also showed positive effects in lymphocyte cell culturing. Results of extraction with solvent methanol showed the highest antioxidant activity. Experimental plant is potential source of natural antioxidants.

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#### EXPRESSION OF EPITHELIAL AND NEURON SPECIFIC CELL ADHESION GENES IN SF767 GLIOBLASTOMA CELLS TECHNIQUES

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Cancer is one of the major causes of death worldwide and most important reason this high mortality rate is the metastasis in which cells break their connection from their primary site of origin and reach the distant parts of body, develop new adhesion junctions their and invade in surrounding tissue. Cell to cell and cell to matrix adhesion has a crucial role in cancer development and metastasis in glioblastoma (an invasive type of brain tumor). This adhesion is due to different genes such as E-cadherin, N-cadherin, Ep-CAM, N-CAM and integrin which express at different time points. Complete cell to matrix attachment (*in vitro*) is achieved within 24h and during this time all these genes undergo a unique pattern of expression. In our study the pattern of expression of these genes during 24h at different time points (0h, 1h, 3h, 6h, 12h and 24h) was estimated in SF767 cell line (a glioblastoma cell line) using both colorimetric quantification and real time PCR techniques and results were compared with the visual observations obtained from photographs of cell culture at 0,1,3,6,12 and 24h. Expression of epithelial specific and neuron specific genes in SF767 cell line was estimated and it was observed that being a brain cell line SF767 was supposed to express neuron specific genes but it was

expressing epithelial genes at significantly higher level than neuron specific genes. Most of the genes had highest expression level at 6h which was consistent with our visual observations that almost maximum cells were attached to the surface of culturing plate after 6h. Both N-cadherin and E-cadherin had maximum expression at 6h and both these genes are responsible for strong cell to cell adhesion but E-cadherin expression was much higher than N-cadherin. Same pattern was observed for Ep-CAM but N-CAM had highest expression at 1h and 24h while at 6h it's expression was almost negligible which give important information about epithelial to mesenchymal transition of cells. All these genes have complex cross talk among each other and this complex communication allows cancer cells to detach from primary site of tumor and attach to the secondary site of tumor.

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#### **EFFECT OF ETHANOLIC EXTRACT OF *CISSUS QUADRANGULARIS* ON THE GROWTH OF MDBK CELLS**

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In many parts of the world, medicinal plants have been used as treatments for human diseases for thousands of years. The herbal products are safer than synthetics that are unsafe to the human and environment. Medical plants play an important role in the management of diseases in developing countries where resources are meager. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds. It is of note that some bioactive compounds present in plants have been reported to interfere with drug kinetics and to produce adverse effects related or unrelated to their pharmacological actions, such as allergic reactions, mutagenic and carcinogenic effects, and several other toxic effects. The increase in number of users as opposed to the scarcity of scientific evidences on the safety of the medicinal plants, have raised concerns regarding toxicity and detrimental effects of these remedies. So the present study was conducted to study the effect (either positive or negative) of *Cissus quadrangularis* (CQ) which is considered a versatile medicinal plant in both Ayurvedic and modern drug development areas for its valuable medicinal uses. The root and stem extracts of the plant *Cissus quadrangularis* have therapeutic efficacy and are known to possess antioxidant, antimicrobial activity, and are routinely used to accelerate the process of bone fracture healing. Its effect was studied on MDBK cell line in order to provide a baseline data for its effect on normal cells and to determine its concentration which is safe and useful as anti-inflammatory agent in the treatment of kidney amyloidosis. To the best of our knowledge, this is the first report that analyzes the effect of CQ on the growth kinetics of MDBK cell line. The present study is employed to delineate the effects of ethanolic extract of CQ on the growth kinetics of MDBK cells. For this cells were counted after every alternate day till seven days and a graph was plotted between cell number and days under different



concentrations of CQ. Then cytotoxic effects were checked by Neutral red Assay. Genotoxic effect was assessed by alkaline comet assay. Cell proliferation was quantified by BrdU assays. The study of growth curve under different concentrations of CQ did not differ significantly under low treatment level (*i.e.*, lower doses of CQ 0.1, 1, 10, 25 µg/ml) but there is significant difference at the 50 µg/ml concentration of CQ. And we find that this concentration has also cytotoxic effects on MDBK cells. The results of Comet Assay reveal no genotoxic effect. We concluded that lower doses of CQ might be useful as anti-inflammatory agent to prevent inflammation in various kidney diseases.

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#### **EFFECT OF ARSENIC, CADMIUM AND LEAD ON PROLIFERATION OF PLACENTAL CHORION CELLS**

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Heavy metals present in environment can effect human health in different ways. These heavy metals can even cross placenta and cause harm to developing fetus. In the present study we investigated the anti-proliferative effects of arsenic, cadmium and lead on human placental chorion cells (PCCs). The cells were isolated by explant method from placental tissue. Anti-proliferative effects of arsenic, cadmium and lead were tested by neutral red uptake assay. Both arsenic and cadmium proved to be very toxic for PCCs. There was marked decrease in cells proliferation when cells were exposed to different metal concentrations for 24 hrs. There was reduction in proliferation of cells on exposure to lead but the effect of lead on PCCs was not as server as that of arsenic and cadmium. Arsenic, cadmium and lead are very toxic for PCCs so there is need to adopt proper measures to reduce the exposure of animals and human.

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### 3. GENETICS

#### X-STR POLYMORPHISM IN SOME ETHNIC GROUPS OF HAZARA REGION, PAKISTAN

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X-STR polymorphism of five ethnic groups of Hazara region, northern Pakistan is reported here. The ethnic groups included; Gujjar, Kerlali, Kohistani, Swati and Tanoli. DNA was extracted from saliva samples of 30 individuals, 15 male and female, of each ethnic group and were typed with 5 X-STR markers using 5 pairs of primers with one primer fluorescently labeled of each pair. The markers included DXS-101, DXS-6789, DXS6809, DXS-7132 and HPRTB. The PCR amplified products were run on ABI-3100 machine and analyzed using GeneMapper software version 4.0. The forensic efficiency of STRs based data was tested for kinship with respect to PD<sub>m</sub>, PD<sub>i</sub>, PIC, Het, GD, LD and PE, using Arlequin software version 3.1. The number of alleles per marker, scored across all the whole populations was 12, 14, 8, 12 and 7 for DXS-101, DXS-6809, DXS-7132, DXS-6789 and HPRTB respectively. The PE and Het were in the range of 0.194 for DXS-6789 to 0.555 for DXS-7132, whereas HET<sub>f</sub> ranged from 0.5075 for DXS-6789 to 0.7761 for DXS-7132. The value of PD<sub>f</sub> ranged from 0.900 for HPRTB to 0.903 for DXS-7132 while PD<sub>m</sub> ranged from 0.736 for HPRTB to 0.831 for DXS-6789. The high value of Power of Discrimination in males and females supported the importance of X-STRs multiplex in forensic identity testing. The least discriminating locus was HPRTB. The genetic distance analysis based on allele frequencies showed that Kerlali and Tanoli people are nearer to one another. Both of these ethnic groups have phylogenetic affinities with Swatis, whereas the Gujjar and Kohistani people has retained their individuality as distinct races.

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#### DESCRIPTIVE GENETIC EPIDEMIOLOGY OF TALIPES IN CASES ASCERTAINED FROM VARIOUS REGIONS OF PAKISTAN

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“Talipes” is a term used to describe congenital deformity of foot and is characterized by the twisted position of ankle, heel and toes. The most commonly occurring form of talipes is ‘talipes equinovarus’ (TEV) or ‘clubfoot’ in which foot

appears to be in downward and inside orientation. It is presented with the fixed cavus, adductus, varus and equines positions. The etiology of talipes remains unclear and there are various risk factors attributed with it. Not much scientific data are available on TEV in the Pakistani population. The present study therefore, attempts to investigate the epidemiological and phenotypic attributes of TEV in the Pakistani cohort. This cross-sectional study included the recruitment of 123 cases of TEV from tertiary care hospitals of Pakistan. The subjects or their parents voluntarily gave their consent for participation and the information regarding demography, phenotypes and clinical aspects were obtained. These analyses showed that there was maximum representation of children with age  $2.26 \pm 1.4$  years among the cohort (60%); the representation of first born subjects was higher (30%). Sporadic cases comprised of 70% of the cases and males predominated the females (M:F ratio; 2.15:1). Majority of the cases belonged to the low economic class (84%). Young maternal age ( $\leq 29$  years) at the time of index subject's birth was observed in high proportion (61%). Among the familial cases, the difference in the affected male-to-female ratio was most striking in subjects with one affected sibship and this difference appeared to decline significantly as the number of affected sibships increased. Bilateral presentation was observed in 63% cases of which 83% were symmetrical. Parental consanguinity was observed to be an insignificant risk factor. The current descriptive epidemiological study is the first step in the determination of potential risk factors associated with this anomaly in the Pakistani population; which will ultimately lead to the better understanding of the etiology and would subsequently allow for the proposal of approaches for its cure and prevention. Genetic/molecular analysis should be performed particularly for the familial cases to identify the causative factors responsible for the disruption in normal limb developmental pathway that would eventually be helpful for the prognosis and treatment.

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#### **ASSOCIATION OF SNP rs10757274 WITH CORONARY ARTERY DISEASE IN LOCAL PAKISTANI POPULATION**

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The present study aimed to investigate the association between a SNP rs10757274 (present at locus 9p21 in the gene for CDKN2B-AS1) and coronary artery disease (CAD) in local population of Pakistan. Allele specific PCR based strategy was used for the identification of genotypes. A total of 350 samples were used for the investigation. Out of which, 220 samples were CAD patients and 130 samples were normal healthy individuals. Effects of parameters like family history of CAD, smoking, presence of diabetes and hypertension in changing the chances of CAD were studied. A strong association was observed between the CAD and factors like smoking (OR: 1.666; 95%CI: 1.042 - 2.664), presence of hypertension (OR: 26.55; 95%CI: 15.95 - 44.20),

diabetes (OR: 3.009; 95%CI: 1.841- 4.920) and the family history of CAD (OR: 4.9; 95%CI: 2.965 - 8.099). Results for the association between the genotype on the basis of rs 10757274 showed a strong association between the GG genotype and occurrence of CAD (OR: 9.603; 95%CI: 5.746 -16.05). The present results suggest the importance of this locus in modulating the chances of CAD in local population of Pakistan.

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**GENETIC EVALUATION OF LADY AMHERST *CHRYSOLOPHUS AMHERSTIAE*, GOLDEN PHEASANT *CHRYSOLOPHUS PICTUS* AND YELLOW GOLDEN *CHRYSOLOPHUS PICTUS* ON THE BASIS OF RAPD MARKERS IN DHODIAL PHEASANTRY, KP, PAKISTAN**

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The present research was carried out to distinguish three species of pheasants at molecular level. Feathers samples were collected from three selected pheasants species present at Dhodial Pheasantry Mansehra and were carried to the Laboratory of Genetics Department Hazara University Mansehra for further analysis. Fourteen RAPD Primers were used in pairs over the nine samples of three pheasant species. On average 3.35 alleles were amplified and the average genetic distance estimated was 7%-63%. Nine samples of three species were clustered into two groups using dendrogram. Group A comprised of samples of Lady Amherst and Golden pheasant while group B comprised of Yellow Golden. It is concluded that the Lady Amherst and Yellow Golden are most distantly related, so the crossing of these two species is recommended for creating maximum genetic diversity.

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**ASSOCIATION OF rs5370 (GENETIC VARIANT OF EDN1 GENE) WITH CORONARY ARTERY DISEASE IN LOCAL POPULATION OF PAKISTAN**

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The present study aimed to investigate the impact of rs5370 (variant of the EDN1 gene) polymorphism on the risk of coronary artery disease (CAD) in local population of Pakistan. A case-control study, involving 320 samples, was accomplished for this purpose. Allele specific PCR based strategy was used for the identification of different genotypes for this genetic variation. PCR amplification results were obtained for 314

samples. Six samples did not give amplification results. Two groups were categorized on the basis of presence of coronary artery disease. The group of patients was named as CAD group whereas the normal individuals were placed in group called as Normal. The results show that frequency of T allele was high in CAD patients. The SNP rs5370 was strongly associated with CAD in local population ( $p < 0.01$ ). T allele was found to enhance the risk value of CAD. TT genotype increased 8 times the risk of CAD as compared to other genotype (OR; 8.8188, 95% CI 5.207 - 14.93,  $p < 0.01$ ). On the basis of this study, it can be concluded that rs5370 is strongly associated with CAD in local population of Pakistan. This also proves that genetic variations of EDN1 may play important role in the on-set of coronary artery disease.

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#### GENETIC ANALYSIS OF COMMON LEOPARD (*PANTHERA PARDUS*) IN AZAD JAMMU AND KASHMIR

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The Common leopard (*Panthera pardus*) is adoptable big cat belong to Felidae family (Nowell and Jackson, 1996) which is among the critically endangered species in Pakistan (Sheikh and Molur, 2005). The study was carried out to analyze the genetic diversity of common leopard from six different populations, so that it can be used as a measure to judge of long-term population health and the ability of common leopard to develop properly with changing environments, that potentially requiring conservation attention. The skin and hair samples were sourced by University of AJK contacts. The obtained samples were categorized into seven different populations of AJK, that includes; Distric Muzaffarabad (pop-1), Distric Neelum (pop-2), Distric Hatian (pop-3), Distric Poonch (pop-4), Distric Bhimber (pop-5) and Distric kotli (pop-6). The DNA was extracted using three different protocols and phenol chloroform triton modified method proved to be the best in terms of producing good quality and quantity of DNA for further molecular analysis by PCR-based SSR markers (FCA043, FCA026, FCA075 and FCA090). Allelic frequencies, genetic diversities (within and among populations) and homogeneity of allele frequencies were calculated with POPGENE 32 program in haploid analysis domain. The highest allelic frequency (1.00) was detected at all loci, in Pop-6 (Kotli). A high proportion of polymorphism (75.00%) was shown by the Pop-1 (Muzaffarabad) and Pop-4 (Poonch). Highest genetic distance (0.4275) was established between the Kotli (Pop-6) and Hatian (Pop-3) populations, and lowest between Hatian (Pop-3) and Neelum (Pop-2) populations (0.0393) of common leopard. Nei's genetic similarities between different populations was highest (0.9614) between of Hatian (Pop-3) and Neelum (Pop-2) population while lowest (0.6521) between kotli (pop-6) and Hatian (pop-3) populations. Higher level genetic differentiation between populations supports our hypothesis of habitat fragmentation.

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### GENETIC DEPENDENCY OF ZIP3 PROTEIN SUMOYLATION AND PHOSPHORYLATION

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Zip3 protein yeast meiosis specific protein belongs to ZMM family protein. The ZMM group protein shifts the resolution Holliday junction towards cross over formation. Here we showed that Zip3 is sumoylated and phosphorylated. We checked this post-translation modification in various in various mutant backgrounds, which indicated that this modification of Zip3 protein depends upon the DNA double strand breaks and *zip1* and *mek1* gene mutant background. This study also revealed that Zip3 is possible substrate for Cdc28 kinase as Mass spectrometry indicated many phosphorylated S/T amino acids residues in Zip3 protein.

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### GENETIC STRUCTURE OF *CHRYSOMMA ALTIROSTRE* REVEALED BY PCR-BASED TECHNIQUES

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Jerdon's Babbler is a globally threatened, restricted range species and an endemic bird to Pakistan. There is no genetic information available for the species so a study was earmarked to reveal the genetic structure of the species, in order to check its future productivity. There we conducted microsatellite analysis. Where, out of 22 microsatellite primers applied, 17 (77.2%) showed score-able amplification results after PCR. On the other hand, 10 (58.8%) microsatellites depicted monomorphic results. While other 7 (41.2%) showed polymorphism in their results. These SSR markers were analyzed through PCR and checked for the results after running the PCR products on Poly-Acrylamide Gel Electrophoresis (PAGE). Amplification of the genomic DNA from each of the 11 genotypes using all the 17 Microsatellite primers revealed a variety of SSR patterns. Out of these 17 primers, seven showed stable pattern with a set of major DNA fragments and were the most effective in detection of genetic polymorphism in the studied populations of *Chrysomma altirostre*. According to the genetic similarity dendrogram constructed for all 30 traits for eleven genotypes detected using 7 polymorphic microsatellite primers.

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### **DETECTION OF POLYMORPHISM A919G AND MUTATION G1255A IN FSHR GENE BY PCR-RFLP IN PAKISTANI INFERTILE**

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Infertility is the major health problem. The causes of infertility include both genetic and non-genetic basis. Less information is available about genetic causes of infertility in Pakistani population as often preliminary diagnosis of the cause artificial reproduction technique (IVF or ICSI) is suggested on infertility clinics. Apart from Y chromosome deletion affecting sperm production and CFTR gene mutation affecting both male and female, FSHR gene mutation and polymorphisms are also associated with male and female infertility. A total 120 subjects were selected where 60 were infertile male (21) and female (39) with various infertility causes and 60 were control subjects who were healthy and fertile. People of age group 31-40 years were found to be highest percentage visiting infertility centers. Follicle stimulating hormone (FSH) levels were recorded and no correlation was found with age. One mutation G1255A and one polymorphism A919G were selected. The exon 10 of FSHR gene was screened for mutation G1255A by PCR-RFLP and polymorphism A919G by nested PCR-RFLP. In present study, none of the subjects harbored this reported mutation in FSHR gene. All three variants (AA, AG, GG) of the polymorphism A919G were observed in the following frequencies. 43.3% of the total patients were homozygous for AA, 50% were heterozygous for AG and 6.7 % were homozygous for AA. 66.6% of the controls were homozygous for AA, 26.6% were heterozygous for AG and 6.8% were homozygous for GG. The data was statistically analyzed by Hardy-Weinberg Equilibrium and chi square for gene and genotype frequencies.

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### **GENETIC POLYMORPHISMS IN ADIPONECTIN AND INSULIN RECEPTOR GENES IN PAKISTANI WOMEN WITH POLYCYSTIC OVARY SYNDROME**

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Polycystic ovary syndrome (PCOS) is the major cause of anovulatory infertility in females. It is a common metabolic and endocrine disorders. The genetic basis of PCOS is not well understood. Current study investigates the possible genomic variants associated

with PCOS in Pakistani women from the Punjab region. As adiponectin possesses insulin sensitizing and anti-inflammatory properties, it has key roles in regulating female reproductive functions. Insulin stimulates ovarian androgen production that affects ovarian steroidogenic responses to luteinizing hormone and follicle-stimulating hormone. DNA samples from ninety-six genetically unrelated PCOS patients and ninety-six control were analyzed by direct sequencing to determine the polymorphisms of different loci on adiponectin (ADPOQ) and insulin receptor (INSR), genes. Significant associations were observed within the genotype frequencies, allele frequencies and multi-SNP haplotype analysis of most of the genetic polymorphisms studied. This study identified new SNPs at position + 349 A/G in ADIPOQ and +1638 T/C in INSR genes. The strong  $r^2$  value suggests that polymorphisms in the ADIPOQ gene were in linkage disequilibrium. In seven major haplotypes of ADIPOQ gene the TGA, TTA and GGG are more frequent in the PCOS group, while the haplotype GGA was found only in the control group. In INSR gene much stronger associations were observed when the three SNPs were considered together as hplotypes. The estimated haplotype frequencies were compared between PCOS and control, the most common haplotype CGT, CAC and CAT was found to be more prevalent in the PCOS. Our study provides evidence of statistically significant association between susceptibility to PCOS in Pakistani women and above mentioned gene polymorphisms. The genetic heterogeneity test showed a strong significance ( $P \leq 0.001$ ) between PCOS and control. This suggests that the susceptible loci for PCOS lie within or very close to the chromosomal regions spanning these genes.

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#### 4. HUMAN AND ANIMAL DISEASES

##### CLINICAL, EPIDEMIOLOGICAL AND VIROLOGICAL FEATURES OF DENGUE VIRUS INFECTIONS IN KHYBER PAKHTUNKHWA PATIENTS PRESENTING TO PRIMARY CARE FACILITIES WITH ACUTE UNDIFFERENTIATED FEVER

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During our study we had visited different areas of Khyber pakhtunkhwa (Swat, Buner, Shangla, Mardan, Malakand, Peshawar, Chitral, and Dir) the dengue infection rate was high in Swat (50%), Shangla (28%), Buner (15%), Shangla (4%), Dir (2%), Mardan (1%) and Peshawar (1%). The infection rate was less in children. The death rate was 92 (0.57%) and in 20-40 years of age the infection rate was much high. The characteristics symptoms were vomiting, splenomegale and abdominal pain. The rate of primary infection was (85.24%) and secondary infection was (14.76%). All the patients improved over a period of 8-15 days.

##### GENOTOXIN/NON GENOTOXIN-INDUCED HEPATOCARCINOGENESIS AND ITS PREVENTION BY SOME ANTICARCINOGENS

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Present study was designed to investigate the hepatoprotective effects of colchicines (0.1 mg kg<sup>-1</sup>b.w) and allyl disulfide (5 mg kg<sup>-1</sup>b.w) against lead nitrate (8 mg kg<sup>-1</sup>b.w) and 1,4-dioxane (10 mg kg<sup>-1</sup>b.w) induced carcinogenic changes in Balb C mice. The study included the activity of enzymes (ALAT, ASAT and LDH) in plasma and liver and some biochemical components (total protein, soluble protein, cholesterol, DNA and RNA) in liver. The biochemical observations were supplemented with histopathological examination of liver sections. Lead nitrate treatment resulted in significant decrease in LDH activity in both plasma and liver, while ALAT and ASAT activities increased in liver but remain unaltered in plasma. Among biochemical components soluble protein and RNA were significantly increased but the activity of total protein, cholesterol and DNA remained unaltered by lead nitrate treatment. Administration of 1,4-dioxane resulted in significant increase in level of both hepatic and serum ASAT, while ALAT

level significantly increased in liver only. LDH level in both liver and plasma, while ALAT level in plasma remained unaltered. Among the biochemical components soluble protein and RNA increased significantly while total protein, cholesterol and DNA remained unaltered by 1,4-dioxane treatment. Colchicine post treatment markedly prevented the lead nitrate induced decrease in the level of LDH in both plasma and liver and ALAT, ASAT, soluble protein level in liver but failed to abolish lead nitrate induced increase in hepatic RNA. Post treatment of allyl disulfide prevented 1, 4-dioxane induced changes in ASAT level in plasma and liver but failed to abolish ALAT level in liver. Histopathological examination of the liver tissues supported the hepatoprotection by colchicine and 1, 4-dioxane. This study demonstrated that lead nitrate and 1, 4-dioxane caused significant carcinogenic and necrotic changes in liver and colchicine and 1,4-dioxane showed little protection. Further study is recommended to investigate their detail anticarcinogenic role against liver damage and hepatocellular carcinoma.

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#### **A STUDY ON PREVALENCE OF BREAST CANCER IN PRE AND POSTMENOPAUSAL WOMEN VISITING INMOL HOSPITAL, LAHORE**

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Breast cancer is a type of cancer originating from breast tissue, most commonly from inner lining of milk ducts or the lobules that supply the duct with milk. This study was designed to evaluate the association of menstrual cycle, reproductive factors, demographic factors, socioeconomic status and family history with breast cancer risk and the influence of these factors on breast cancer incidence. Data was collected from INMOL hospital, Lahore. Information collected by questionnaire form 150 women during their treatment between ages 25 and 80. Breast cancer is affecting both pre and postmenopausal women but its increased risk was observed in premenopausal women about (56.67%). Earlier age at menarche (<13) was associated with increased risk of breast cancer among both pre- and postmenopausal women, while women who got married at early age of 20-24 years were at higher risk, first birth at age of 20-24 years and menopause at age of 45-49 years were also at high risk. Whereas greater BMI in postmenopausal women was associated with greater risk of breast cancer than in premenopausal women and low socioeconomic status was also associated with high prevalence of breast cancer risk both in pre- and postmenopausal women. Family history was observed to be less related to this study. Our study concludes that menstrual, reproductive, demographic factors, socioeconomic status and family history among women have contributed to the recent increase in breast cancer incidence, particularly in young women.

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**ASSESSMENT OF RENAL FUNCTION PARAMETERS AMONG ISCHEMIC STROKE PATIENTS IN OUR LOCAL POPULATION**

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Renal impairment has been linked to higher stroke risk. Renal dysfunction in first-ever ischemic stroke, however, has not been investigated thoroughly. The present study was conducted to investigate renal functional status in ischemic stroke, to assess the prevalence and to determine whether decreased kidney function is a risk for first-ever ischemic stroke. In this cross-sectional study 150 subjects were divided into two groups, Ischemic stroke group (n=100) and Control (n=50). Ischemic stroke group had a clinical diagnosis of ischemic stroke confirmed in hospital and were admitted within 48 hours of symptom onset. Control group comprised of healthy individuals from the same population. Direct questioning was done to assess the prevalence of risk factors in the population. Kidney function was estimated using serum creatinine and blood urea along with eGFR calculated by MDRD equation. Kidney dysfunction was defined as eGFR of <60 ml/min/1.73m<sup>2</sup>. Significant difference in mean serum creatinine (2.41 mg/dl vs 0.93mg/dl), blood urea (67.3 mg/dl vs 34.6 mg/dl) and eGFR (54.62ml/min/1.73m<sup>2</sup> vs 85.90 ml/min/1.73m<sup>2</sup>) was observed between ischemic stroke patients and controls. The prevalence of eGFR <60 ml/min/1.73m<sup>2</sup> in patients with stroke was 63%, significantly higher than in controls. Moderate to severe reduction of eGFR in patients with ischemic stroke indicated renal impairment and kidney dysfunction. In ischemic stroke patients, low estimated glomerular filtration rate is associated with increased risk of first-ever ischemic stroke.

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**MOLECULAR EPIDEMIOLOGY OF HEPATITIS B VIRUS (HBV) GENOTYPES AMONG PATIENTS WITH CHRONIC INFECTION IN SOUTHERN BELT OF KHYBER PAKHTUNKHWA, PAKISTAN**

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Hepatitis B virus (HBV) is the global health problem and about 5% of the world population has been infected with HBV with approximately 400 million peoples are chronic carriers. Pakistan falls in the highly endemic region for HBV. HBV genotypes in chronic HBV patients in the southern belt (Dera Ismail Khan (D.I. Khan) Division, Bannu Division and Kohat Division) of Khyber Pakhtunkhwa Pakistan were identified from June 2010 to March 2012. A total of 300 blood samples were collected from HBV

suspected individuals of either sex, with age group ranged from 1-70 years. All the subjects were screened with Immunochromatographic test (ICT), for HBsAg. The positive patients for HBsAg were processed for the detection of HBV DNA by nested PCR and HBV DNA positive patients were subjected for HBV genotyping by genotype specific PCR. Complete Blood Count (CBC) and Liver function tests (LFTs) were performed for every individual and were compared with particular HBV genotype. Data obtained was analyzed with statistical software SPSS. Out of the total 300 samples analyzed, 245 (81.67%) showed genotype specific bands and were classified for various genotypes (A, B and A+B) while the remaining 55 (18.33%) were untypable. The HBV infection in this study in HBsAg positive patients were attributed predominantly to viral genotype D constituted 115 (38.33%) of the total individuals. Genotype A was the second prevalent with 95 (31.67%) and mixed genotypes A+D were detected in 35 (11.67%) samples, while genotypes B, genotype C and genotype F were not found in this study. The highest prevalence of genotype A (55%) was found in Kohat Division and that of genotype D (60%) and mix genotype A+D (15%) in D.I. Khan Division. The prevalence of genotypes was assessed further with respect to patient's age. The high frequency of all genotypes, A (36.84%), D (43.5%) and A+D (42.9%) was found in the age group of 16-30 years. However, in individuals aged more than 60 years, only genotype A 10 (10.53%) was found and no other genotype, mixed genotype or untypable genotype was found in this age group. Proper vaccination and treatment strategies should be initiated to stop the spread of this dreadful disease.

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### VECTOR-BORNE INFECTIONS IN PAKISTAN

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Infections with vector-borne pathogens are a major source of emerging diseases. The ability of vectors to bridge spatial and ecological gaps between animals and humans increases opportunities for emergence. Vector-borne diseases especially insect-borne diseases have devastated human populations in the past and continue to be an important cause of severe morbidity and mortality. Many diseases are re-emerging in developing countries due to economic problems and the resistance of vectors to insecticides and of pathogenic organisms to therapeutic drugs. Fortunately, Pakistan is free of several dreadful vector-borne diseases like Yellow fever, Filariasis, Trypanosomiasis, Onchocerciasis (river blindness), Schistosomiasis (Bilharziasis), Japanese B encephalitis (JBE), etc. The three most important vector-borne diseases in Pakistan are Malaria, Dengue and Leishmaniasis, the latter two became more prevalent probably due to cessation of malaria eradication program. Less prevalent vector-borne diseases in Pakistan include, West Nile Virus (WNV), sandfly fever, CCHF, etc. Complexity of epidemiology and adaptive plasticity of pathogen and arthropod make the vector-borne

diseases especially difficult to control. Vaccines are unavailable for all but a few diseases; and even when they are available, as for YF, prevention can be difficult to achieve. Tools for treatment are scarce. By their very nature, protozoa are more complex organisms than bacteria and viruses, with more complicated structures and life cycles. This presents problems in vaccine development. Malaria is a major cause of morbidity and mortality. Immunity is acquired but is suboptimal, being slow to develop and incomplete. With data from Phase III trials indicating that the leading malaria vaccine candidate, RTS, S, has limited efficacy, it is necessary to reconsider approaches to the development of a vaccine capable of inducing long-lived protection. Recent studies on additive, synergistic or antagonistic effect of several constituents of *Artemisia annua* indicate that artemisinin-B is more potent antimalarial than artemisinin. The aqueous extract of *Artemisia annua* is several times more potent than pure artemisinin. Despite significant advances in the search for potential dengue vaccines and new therapeutic schemes for malaria, the control of these diseases remains difficult. The interruption of transmission still relies on vector control measures that are currently insufficient to curtail the cycle of epidemics. Deficiencies in our knowledge of vector biology hinder public health efforts for vector control. Challenges of dengue, malaria and leishmaniasis control warrant concerted research efforts. Socioeconomic development, though difficult & expensive, is perhaps the most effective control measure for all public health problems including vector-borne disease.

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#### **CLUSTERING OF MODIFIABLE CVD RISK FACTORS IN ADULTS AGED <50 YEARS, IN DISTRICT HYDERABAD, PAKISTAN**

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Heart disease is the primary cause of mortality all over the world. Millions of peoples die each year due to Cardio Vascular Diseases (CVDs). CVD disease is a silent killer; it cannot be identified until it reaches at its severe stage. CVDs can be predicted and even prevented if CVD factors are known before the diseases take place. CVD risk factors are physical inactivity, unhealthy diet, obesity, hypertension, hypercholesterolemia, and diabetes, and these are increasing at alarming rates and many individuals remain unaware, undiagnosed and untreated. A population based, cross-sectional survey was conducted on 332 adults 198 male and 134 female aged 30 to 49 years from randomly selected communities in Hyderabad, Pakistan. Only apparently healthy individuals were included in the study and all those suffering from any CVD disease or on smoking and any other drug use were excluded from this study. Out of total number of 332, 81 (24.39%) had higher number of risk factors and these risk factors were higher men 59 (29.79%) for developing CVDs than women 21 (15.67%). This study also indicates that these CVD risk factors increase with age. From 30-39 the prevalence of

maximum risk factors were 30 (13.51%), and from 40 to 49 the prevalence of these risk factors was 52 (47.27%), which suggest that these risk factors increase with age. Men were at more risk (OR. 1.9, 95% CI 1.1-3.2,  $P < 0.02$ ) than women, and age group 40-49 years was at higher risk (OR. 5.7 95% CI, 3.3-9.8,  $P < 0.0001$ ) than the age group from 30-39 years.

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### COMPARING CONVENTIONAL AND MOLECULAR ASSAYS IN DETECTING MALARIAL INFECTION

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The reliability and sensitivity of PCR helps differentiate between false positive and false negative results. Experiments were carried out to compare sensitivity of PCR protocol with conventional blood smear microscopy. Of 60 samples collected from malaria suspected patients, 18 (30%) were found positive when examined through blood smear method, however, when same blood samples were subjected to PCR, only 5 (8.33%) samples were found positive via gel documentation by visualizing plasmodium DNA bands at 801bp. Further studies, aimed at molecular taxonomy of various malarial isolates/strains are under way. PCR-based techniques are a recent development in the molecular diagnosis of malaria, and have proven a most specific and sensitive diagnostic methods. Microscopy is an established, relatively simple technique that is familiar to most laboratories. Present study was carried out to detect plasmodium, the causative agent of malaria from 100 samples collected from different areas of district Umerkot and Tandojam by PCR and conventional blood smear examination.

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### STUDY OF SOME INFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES IN THE MALARIAL PATIENTS

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The present study investigated the serum levels of inflammatory and anti-inflammatory cytokines in malarial and healthy individuals. Tumor necrosis factor alpha (TNF- $\alpha$ ) and interferon gamma (IFN- $\gamma$ ) were studied as inflammatory cytokines. The interleukin-10 (IL-10) & transforming growth factor beta (TGF- $\beta$ ) levels were detected as the anti-inflammatory cytokines. Eighty blood samples were used for the study; out of which 32 samples were taken from normal healthy volunteers and 48 from malarial patients. Samples from the patients of malaria were further differentiated on the basis of detected species of *Plasmodium*. One group was having *P. falciparum* as a causative

agent, while the other group was carrying *P.vivax* parasite in the blood. The results showed that IL-10 levels were higher in malarial patients as compared to the healthy individuals ( $02.68 \pm 01.67$ ). No significant difference was observed between patients suffering from *P.falciparum* ( $91.00 \pm 33.63$ ) and *P. vivax* ( $88.95 \pm 14.67$ ) for this cytokine. The levels of IFN- $\gamma$  were found to be increased in the malaria patients as compared to healthy individuals ( $28.57 \pm 12.84$ ). TGF- $\beta$  levels were elevated in malarial patients as compared to healthy individuals ( $15.66 \pm 01.70$ ). Difference in TGF-  $\beta$  levels were not significant between the patients suffering from *P.falciparum* ( $22.51 \pm 04.59$ ) and *P.vivax* ( $24.23 \pm 04.35$ ). The level of IFN- $\gamma$  was higher in *P. vivax* ( $74.96 \pm 31.70$ ) as compared to healthy group, however the difference was not significant between the IFN- $\gamma$  levels in *P.vivax* and *P.falciparum* ( $72.07 \pm 10.21$ ). No difference was observed between *P. vivax* ( $50.95 \pm 27.82$ ) and *P. falciparum* ( $35.18 \pm 26.35$ ) for TNF-  $\alpha$ . It can be concluded that both inflammatory and anti-inflammatory cytokine levels alter in human body on exposure of *Plasmodium* parasite.

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#### INTERACTIONS BETWEEN MORTALITY AND MORBIDITY RATE IN PROSTATE CANCER PATIENTS FROM FAISALABAD DISTRICT

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The determination of mortality and morbidity of prostate cancer patients is the main considerations that lead to know the conditions and surviving rate among them. This idea is different and recent from other previous researches. Many researchers only studied the single treatment effect while in this article comparison of different recent treatments has been observed. Specific diagnostic tests has been studied in old research articles while almost all type of diagnostic tests that are used in PINUM hospital Faisalabad has been taken for comparison . Socioeconomic status and smoking of patients has exclusively studies with other parameters and showed significant effect on mortality and morbidity in prostate cancer patients. In this study morbidity and mortality of prostate cancer patients, after one year of diagnosis, was evaluated that was conducted in Punjab Institute of Nuclear Medicine (PINUM) and data of 30 patients, had collected from July 2009 to November 2009. All the patients have been undergone following tests: PSA tests; diagnostic tests that were biopsy and bone scan. The types of treatment that patients had done consist of radiotherapy, surgery, chemotherapy and hormonal therapy. Significant patients of age>60 year died out of 27 ( $P<0.05$ ) as compare to patients of age < 60 years. Comparatively high death rate was observed in low socioeconomic class ( $P<0.01$ ).No significant result has been observed after PSA test in patients. Bone scan

was done in all the patients to check bone metastasis that was observed in 23 patients out of which 18 (78.3%) patient died and showed significant mortality as compared to patients without bone metastasis. One group with PSA level > 20 ng/ml, high death rate was detected among them ( $P < 0.05$ ) as compared to patients with PSA level < 20 ng/ml less mortality detected among them ( $P > 0.05$ ). In the case of smokers and nonsmokers groups, smoking caused more death in smoker group of 19 patients ( $P < 0.01$ ) whereas less patients died in 11 non-smokers patients ( $P > 0.05$ ). Surgery showed less effect on mortality in 19 patients ( $P > 0.05$ ) whereas more mortality observed in 11 patients without surgery ( $P < 0.05$ ). Mortality has been significantly observed in 13 patients that was not exposed to radiation ( $P < 0.05$ ) in contrast 17 patients that have been exposed, radiotherapy has less effective in them ( $P > 0.05$ ). In the case of hormonal therapy, high mortality determined in 8 patients that did not experience therapy ( $P < 0.05$ ) while less treatment effect observed in patients that have been treated ( $P > 0.05$ ). In 19 patients chemotherapy have been used among them no difference has been observed between mortality and survival rate ( $P > 0.05$ ) but 11 patients that remained untreated show high mortality ( $P < 0.05$ ).

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#### **ROLE OF S180L POLYMORPHISM IN MAL/TIRAP ON ETIOLOGY OF MALARIA CAUSED BY *PLASMODIUM FALCIPARUM***

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*Plasmodium falciparum* induced malaria is one of the major causes of human morbidity around the globe. This infection is combated by host immune system where Toll-like interleukin 1 receptor adaptor protein (TIRAP) gene provides Myeloid differentiation primary response 88 (MyD88) adaptors-like (Mal) as an important product in signal transduction pathway for cytokines production. In susceptibility or resistance to malaria, TIRAP S180L polymorphism is proposed to play a role more important than that of any particular *Plasmodium* species and this study aimed to evaluate the same. 73 patients of malaria caused by *Plasmodium falciparum* and 60 healthy individuals were genotyped for TIRAP S180L polymorphism during the current research work. Allele Specific PCR based strategy was used for the identification of different genotypes. It was found that homozygous carriers for Ser180 allele (CC) were at a 0.301 times reduced risk of malaria due to *P. falciparum*. Leu180 allele in homozygous condition (TT) made the individual 2.946 times more susceptible to this infection. Heterozygous condition (CT) did not modulate the chances of malaria induced by *Plasmodium falciparum*. A total of 41 samples from both groups showed no genotyping results. A detailed investigation for the occurrence of alleles other than Ser180 and Leu180 in this population may solve the mystery. On the basis of present results, a strong association may be concluded between the S180L polymorphism and malaria caused by *Plasmodium falciparum*.

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**PREVALENCE OF *SALMONELLA* SPP. IN EGGS OF LAYER HENS IN  
VARIOUS AREAS OF RAWALPINDI DURING WINTER**

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*Salmonella* are one the most important groups of bacteria responsible for disease in poultry and other animals including man. They comprise of over a thousand serotypes but mostly *Salmonella typhimurium*, *S. servora* and *S. enteritidis* are found in poultry. Contamination of eggs can be either by shell surface or by contaminated contents *in vivo*. *S. typhimurium* and *S. enteritidis* infect reproductive tract in chicken and contaminate eggs but *S. enteritidis* persist in the eggs after eggs are laid. The study was conducted in Bacteriological lab of Poultry Research Institute (PRI) to determine the prevalence of *Salmonella* in eggs of layer. 250 fresh eggs were collected from various poultry forms of Rawalpindi during Oct, 2007-Feb, 2008. Only intact eggs (no crack) were included in the study. Different Agar Medias were prepared for the culturing of *salmonella* to check its prevalence in various parts of egg. Only 9 eggs (3.6%) were found to be *salmonella* positive while the remaining 241 (96.4%) were *salmonella* negative. Only two species of *salmonella* i.e. *S. typhimurium* and *S. enteritidis* were found present in the eggs. Both the species were present in albumin as well as yolk. In *salmonella* positive eggs the percentage of *S. enteritidis* was 89% and *S. typhimurium* was 11%. *S. servora* was not found in any sample. The prevalence of *salmonella* in egg shell (78%) was high as compared to other parts (22%) because of direct exposure of eggs to the environment. The prevalence of *salmonella* in egg shells might be a result of transport and prolonged storage in contaminated trays.

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**CHEMOTHERAPEUTIC EFFICACY OF SELECTED DRUGS IN  
COCCIDIOSIS AFFECTED RABBITS**

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The current study was conducted aimed to investigate the comparative efficacy of different chemical and herbal drugs used for the prevention and treatment of coccidiosis in rabbit. A total of 112 rabbits were divided into seven (7) groups; each group was labelled as Healthy control, infected but untreated, infected and treated with Toltrazuril, Sulphadimidine Sodium *Nigella sativa* L. (kalonji seeds), *Nigella sativa* L. (kalonji Oil) and Garlic. Faecal examination was performed carefully by different techniques i.e., direct smear, flotation etc. and the oocyst of *Eimeria* spp. were observed abundantly in rabbits' faecal samples. Quantitative analysis was performed with McMaster technique which gives oocyst per gram (OPG) to determine the severity of infection. The results

were analysed and it was concluded that Chemical drugs minimize OPG (Oocyst per Gram) dramatically *i.e.*, Sulphadimidine Sodium and Toltrazuril showed 48% and 74.4% efficiency in decreasing OPG (Oocyst per Gram) respectively. Herbal drugs *i.e.*, *Nigella sativa* L. seeds (kalonji) in oil emulsion and *Nigella sativa* L. seeds in water suspension minimized the OPG (Oocyst per Gram) slowly 57% and 53.4% oocysts respectively in 14 days. Garlic showed 57.8% decreased oocysts in faeces.

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#### DETECTION AND CHARACTERIZATION OF GASTROENTERITIS VIRUSES FROM WATER BODIES OF PAKISTAN

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Sewage contaminations of drinking water or even of recreational water are of serious health concern. Continuous and reliable monitoring of the water resources is therefore crucial for the rapid assessment of health hazards. Presently, bacterial indicators are used for the quality of water but potential viral pathogens remain undetected. In this study we evaluated the civic water resources of highly populated areas of Lahore, Rawalpindi and Islamabad for the presence of Enteroviruses contamination in parallel with sewage water monitoring. Different viral concentration procedures like PEG 6000, ultracentrifugation and cell culture were used to contemplate the highly diluted environmental samples which were eventually detected by RT-PCR analysis. Specific set of primers were used for the rapid detection of enteroviruses from different water samples collected from three cities of Pakistan. The level of viral contamination was quite high which indicate the potential threat of the gastroenteritis in the surrounding areas.

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#### PREVALENCE OF HEPATITIS C VIRUS (HCV) GENOTYPES IN KHYBER PAKHTUNKHWA (KPK) AND PUNJAB PAKISTAN

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Pakistan carries huge burdens of chronic hepatitis and mortality rate due to hepatocellular carcinomas and liver failure. However, prevalence of HCV genotypes in Pakistan (Khyber Pakhtunkhwa and Punjab) is currently not available. The objective of

current study was to determine the prevalence of hepatitis C virus (HCV) positive subjects and their related genotypes in patients screened at diagnostics lab of Atta-Ur-Rehman School of Applied Biosciences (ASAB), National University of Sciences & Technology (NUST), Islamabad, Pakistan. A total 15000 HCV positive patients were screened for HCV. Patients of either sex, with more than fourteen years of age were included. PCR positive samples were further preceded for HCV genotyping. Samples reactive for HCV RNA were considered positive. Present study demonstrated considerable incidence of HCV in KPK and Rawalpindi, Pakistan. Out of 15000 registered patients, 5580 (43.3%) were male and 9420 (52.7%) were female. Genotype 3a occurs more frequently in HCV patients with rate of 54.44% followed by Genotype 2a with rate of 12.8% whereas, genotype 5a {0.08%} was rare and 5.92 % samples remained untyped. Subtype 3a is the predominant HCV genotype in Pakistan. Presence of untyped samples indicates the possibility of some new genotypes in this area. In this connection, sequencing of these samples can be very helpful.

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#### **EPIDEMIOLOGY OF LEUKEMIA IN A HOSPITAL POPULATION IN DISTRICT FAISALABAD**

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An epidemiological study was carried out in a hospital population of Faisalabad city including Allied Hospital, District Head Quarter Hospital, Sahil Hospital and Punjab Institute of Nuclear Medicine. A standard questionnaire was used to collect information including different parameters like, age, sex, birth order, exact diagnosis, genetic relationships, education, socioeconomic status, smoking, physical activities, allied diseases, locality and family history of patients. The study was aimed to investigate the occurrence and prevalence of leukaemia in a hospital population. Chronic myeloid leukaemia (CML) was most commonly occurring malignancy contributing 42.28 % followed by Acute lymphoid leukaemia (ALL) 26.18 %, Acute myeloid leukaemia (AML) 16.11% and Chronic lymphoid leukaemia (CLL) 15.43 %. There was a significant ( $P < 0.05$ ) gender depicted difference in the prevalence of leukaemia. The patients with blood group O<sup>+</sup> (29.53 %) were more affected with Leukaemia. 4<sup>th</sup> Birth order (16.78%) showed the highest representation of all leukaemia. Rural (62.42 %) population was significantly influenced than urban (37.58 %) population. Patients with low level of education were more affected ( $P < 0.01$ ) with leukaemia in contrast with highly educated patients. Highly significant consequences of consanguinity had been observed among patients and similar result was found for first cousin marriages in contrast to unrelated marriages. Highest percentage of patients among various surnames was observed in Arian (29.53 %) and lowest in Mughal (1.35 %). Smoking was important risk factor for occurrence of leukaemia. 50.34 % patients were smokers followed by non-smokers (42.95 %) and ex-smokers (6.71 %). It was found that 59.73 % patients

belonged to the lower class followed by middle class (35.57 %) and 4.70 % in upper class.

### **SCREENING OF BRUCELLOSIS IN FARM ANIMALS AND RELATED PERSONALS BY USING LOCALLY PREPARED ANTIGEN**

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Brucellosis is a zoonotic disease caused by *Brucella* spp. *Brucella abortus* cause abortions. Weak calves, still births, infertility and characteristics lesions are primarily placentitis, epididymitis and orchitis. In human its effects are multisystem. Brucellosis among human shows many symptoms like scarlet fever, arthralgia or arthritis, sweating with peculiar smell, pain in joints, backache chest pain, weight loss, headache, involuntary limb movement, heart attack, insomnia, limb and back pain and fatigue. Current study was designed to work out prevalence of Brucellosis in domestic cows, buffaloes and associated persons around Lahore. A total of 398 animal belonging to 60 families were screened by milk Ring test to check the presence of *Brucella* antibodies in the milk. The effected animals were detected in the 20/60 families. The prevalence of Brucellosis was 17.58 % in cows and buffaloes. Cows were significantly ( $P > 0.05$ ) more affected than buffaloes. Brucellosis was associated with history of abortions, but non aborted animals also show some degree of infection. All the families dealing with infected animals were screened by using RBPT. Out of 97 members 5 were found to have *Brucella* antibodies. Prevalence of disease is was similar in males and females. Also alteration in RBCs, MCV, MCH, MCHC and PLT was observed. Although the means were not statistically different. S.Bilirubin, SGOT, SGPT and ALP were slightly high in positive members but the differences were not statistically significant. The present study reflects much higher prevalence of Brucellosis ( 17.58 %) around Lahore. This is a serious threat to the public health and to the animal life.

### **CHARACTERISTICS OF REFRACTION IN ANTERIOR CORNEAL DYSTROPHIES PATIENTS FROM DIFFERENT AREAS OF PUNJAB VISITING LRBT HOSPITAL**

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Present retrospective, analytical study was performed between October 2012 and June 2013 on all cases of corneal dystrophies seen from Punjab during ophthalmological consultation at the Layton Rahmatullah Benevolent trust hospital and General hospital.

The aim of this research was to determine the characteristics of refraction associated with anterior corneal dystrophies (ACDs). The techniques used for the diagnosis of anterior corneal dystrophies were an examination of cornea using a slit lamp microscope, topography, orbscan, keratometry, phoropter and far visual acuity. Indeed a drop in visual acuity and blindness were the main reasons for consultation in this study. 53 cases of ACDs were identified from Punja, corresponding to the hospital prevalence of 0.5/1000. ACDs were identified in 13 women and 40 men. ACDs were predominant in age group 11-20 years. Visual disability due to refractive errors is very common in anterior corneal dystrophies. An understanding of pathophysiology of each corneal dystrophy (CDs) will eventually require the development of animal models of disorders. More elaborative studies should be performed concerning refractive errors due to CDs to figure out measures that can aid in decreasing visual disability of affected individuals.

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#### **EPIDEMIOLOGICAL EVALUATION OF THALASSEMIA AT ALI ZAIB FOUNDATION, FAISALABAD**

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The epidemiological evaluation of 1005 thalassemia's patients was performed through standardized questioner at Ali Zaib Foundation, Faisalabad.. The data collected was analyzed for epidemiological and genetical evaluation and pedigree analysis. The male patients (N=580, 57.71%) and female patients (N=425, 42.29%) were non significantly ( $p>0.05$ ) different. Thalassemia patients were splited into thalassemia major (TM) and thalassemia intermediate (TI). The TM was more predominant (93.53%) than TI (6.47%). The sex ratio was 136.47 showing more males affected than females. The potential of affliction for TM and TI was highly significant ( $p<0.001$ ). Mean age of diagnosis was  $(4.25\pm0.131)$  years. The majority of patients (27.96%) were observed between 3-5 years of age groups. The highest prevalence of patients was observed in 3<sup>rd</sup> birth order (32.83%) and lowest in 8<sup>th</sup> birth order (1.49%). Majority (63.89%) patients belonged to lower class followed by middle class (34.13%) and (2%) in upper class. Maximum number of patients was from illiterate parents (Father, N=315, 31.35% and Mother, N=475, 47.27%). Educated families had few affected children. Percentage distribution of thalassemia patients represented 30.54% belonged to rural areas and 69.46% belonged to urban areas. The incidence of thalassemia was significantly ( $p < 0.001$ ) highest in 1<sup>st</sup> cousin marriages compared to unrelated. Occurrence of thalassemia was the highest in outcomes of first cousin marriages (66.87%) than in unrelated (19.11%). The least occurrence of disease was observed in outcomes of bradari relation (2.69%) marriages. Male were highly ( $p < 0.001$ ) affected in 1<sup>st</sup> cousin marriages outcomes compared to female. The coefficient of inbreeding in first cousin was  $F= 0.058$ . Maximum patients were observed in surname Rajpot (21.89%) and lowest in Khokher

(3.68%). Autosomal recessive mode of the inheritance was observed in thalassemia patients.

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**EFFECT OF GINGER (*ZINGIBER OFFICINALE*) ON HYPERGLYCEMIA AND HEMATOLOGICAL PARAMETERS IN ALLOXAN INDUCED MALE DIABETIC RABBITS**

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Several herbal remedies are in practice to treat diabetes. These treatments are considered safe as far as their toxicological effects are concerned. The purpose to design present study is to affirm the anti diabetic effects of ginger extract in alloxan induced diabetic rabbits along with its effect on hematological parameters. Present study unveils the anti diabetic properties of ginger administration in diabetic animals, as there was a significant lowering of blood glucose level in experimental animals. No significant change was noticed in all hematological parameters (TLC, Hb, TEC, platelet count, MCH, MCHC, PCV, neutrophils, lymphocytes, monocytes and eosinophils) However, there was observed an overall tendency of ginger extract to affect the hematological parameters with a way forward to have an extended study of this extract on hematological parameters.

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**IDENTIFICATION OF ARTICULATED PROTEINS IN CANCEROUS BREAST TISSUES VIA USING SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)**

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Pakistan is also facing high burden of breast cancer in females which is the most familiar cause of female's death. In Pakistan, most of the breast cancer diagnosed at III and IV stage. The study was conducted to identify the articulated proteins in cancerous and normal breast tissues. The cancerous and normal breast tissue samples were collected from Allied and DHQ hospitals Faisalabad. The cancerous and normal breast tissue samples were analyzed through Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Quantitative analysis for breast cancerous tissue protein showed increased protein content as compare to normal breast tissue samples ( $0.94 \pm 0.002$  &  $0.85 \pm 0.384$  mg/ml). Qualitative analysis showed that number of protein

bands increased in malignant tissues as compare to normal tissue. The number of bands observed in malignant tissues ranged between 6-15 whereas in normal tissues they ranged between 5-13. The maximum molecular weight of isolated protein observed in malignant tissues was 220 KDa and minimum molecular weight was 10 KDa. However in normal tissue samples the molecular weight of isolated protein ranged between 10-120 KDa. It was observed that 18 isolated proteins of molecular weight ranging between 10-120 KDa were present in both normal and malignant tissue samples. The most of proteins articulated in malignant tissues belong to class of heat shock proteins (70, 60, 80, 84, 27 KDa). It was observed that isolated proteins of molecular weight 24, 32, 77 and 41 KDa were specific to only normal tissue samples whereas proteins of molecular weights 16, 22, 26, 27, 59, 64, 65, 80, 100 and 220 kDa were specific to malignant individuals only. These isolated proteins can be used as biomarker for the early detection of breast cancer.

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#### **PREVALENCE OF CUTANEOUS LEISHMANIASIS IN AFGHAN REFUGEES IN UPPER DIR DISTRICT**

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There are over 45,000 named species of protozoa, living of which nearly 10,000 are parasitic in invertebrates and in almost every species of vertebrates. The protozoa that infect humans ranges from forms that are never pathogenic to those that cause Malaria, Sleeping sickness, Chagas disease and Leishmaniasis, now regarded as being among the major diseases of tropical countries and which together threaten over one quarter of the population of the world. Leishmaniasis is now known to be caused by complex species and subspecies of *Leishmanai*. Cutaneous leishmaniasis is a skin disease cause by *Leishmania topica*. This disease is transmitting by sand fly. The disease is endemic along entire Western border of Pakistan. During the present research, the samples were collected from infected Afghan refugees of different areas of Dir Upper. Total 274 samples were collected among which 169 (62%) were positive cases. Total 112 (66%) males and 57 (34%) females were effected. The high prevalence rate (49.7%) was noticed in the age of 11-20 years old peoples. The prevalence rate between the age of 0-10 was 29.1%. The low prevalence rate (13.6%) was notice in the peoples over 30 year. Mostly one lesion was noticed in an effected people (69%). The patients with two lesions were 22%. The numbers of effected persons with more than two active lesions were little in number i. e. 9%. Most of the lesions were reported on face (36.4%). The second effected part of body noticed in the study was hand (26.5%). Most of the lesions were dry (81%). It is concluded that high prevalence rate was observed. People's awareness is very necessary.

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**EFFECT OF OXOVANADIUM(II) (VO-L2) ON HYPERGLYCEMIA,  
HEMATOLOGICAL AND SEROLOGICAL PARAMETERS OF ALLOXAN  
INDUCED DIABETIC RABBIT**

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Vanadium has been known for its antidiabetic and insulin mimetic properties for about 100 years. Both inorganic and organic compounds have role in treating diabetes however, the organic compounds are considered far better in therapeutic capability, are safer and more absorbable than inorganic vanadium compounds. The objectives of current study were to evaluate the insulin mimetic effects as well as to determine the biological effects of novel organic compound, "Oxovanadium (II) (VO-L2)", on hyperglycemia, hematological and serological parameters in alloxan induced diabetic male albino rabbits. Diabetes was induced by mean of interpretational injections of alloxan. The diabetic treated group was treated with candidate compound by giving oral dose for 5 days. This experiment showed a significant glucose lowering effects in response to candidate compound; moreover a significant increase was found in RBCs count, hemoglobin contents, WBCs count and neutrophil number whereas a significant decrease was found in packed cell volume, mean cell volume, monocyte count, lymphocyte and basophil count. No significant changes were found in MCH, MCHC and eosinophils. Among serological parameters, a significant increase was found in uric acid, SGOT, SGPT and bilirubin and a significant decrease in urea level. No significant changes were found in creatinine. The current study reflects that although the candidate compound has antidiabetic property, side by side it may also have some effects on liver and renal function which suggest that it may not be used for treating diabetes in its current form.

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**PERCENTAGE OF HEMORRHAGIC SEPTICEMIA CARRIER BUFFALOES  
AND THEIR IMMUNITY STATUS AT DISTRICT SWAT, KHYBER  
PUKHTOONKHWA, PAKISTAN**

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Infectious diseases such as bacterial, viral, fungal, protozoan and parasitic are



one of the major problems in farm animals (Radostits *et al.*, 2000). In Pakistan the infectious bacterial diseases found are anthrax, tetanus, blue tongue, black quarter, brucellosis and HS (Afzal, 2009). HS is an acute, fatal disease not only in domestic cattle and buffaloes but also in wild ruminants (Shivachandra *et al.*, 2011). HS is caused by *P. multocida* manifesting an acute and fatal septicemia. The predisposing factors are radical changes in weather such as onset of monsoon, poor health status of the animal due to the nutritional and immunological deficiencies and with an excessive work load in draught animals (De-Alwis, 1999). The present study was conducted to find out the percentage of hemorrhagic septicemia (HS) carrier buffaloes. Pharyngeal/ laryngeal swabs of suspected animals were cultured for the identification of carrier animals of *Pasteurella multocida* (*P. multocida*) while blood samples were processed to determine the antibodies titer against *P. multocida*. Out of 200 buffaloes 8(4%) were identified as carriers of HS. Serological study of blood sample showed that out of 200 buffaloes 156(78%) had antibodies titer  $\geq 1:16$  which were considered immune against HS. All 8 positive carrier buffaloes of HS showed positive immunity against the disease, 4 of them had antibody titer 64, 2 of them had 128 while 1, 1 had antibodies titer 16 and 32 respectively. The pathogenicity test of these isolates showed that they were pathogenic to mice and caused their death within 24 hours of inoculation. It is concluded that animals recovered from HS can act as carriers of this deadly disease and a source of infection for healthy animals.

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#### SEROPREVALENCE OF TOXOPLASMOSIS IN WOMEN OF LOW SOCIO-ECONOMIC STATUS FROM LAHORE

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The protozoan parasite *Toxoplasma gondii* cause toxoplasmosis, it is zoonotic disease that occurs worldwide. During present study, we report the association of low socioeconomic status and seropositivity of Toxoplasmosis in women of Lahore city was reported. ELISA technique was used to determine the IgG antibodies. Serological test was performed on 83 women blood samples, among these 24 % (n=20) women showed specific toxoplasma IgG antibodies and while 76% (n=63) women were seronegative. All the samples were categorized into two age groups. Seroprevalence of Toxoplasmosis were higher (29.4%) in older age group (30-40years), while other group (19-29 years) had only (22%) prevalence, lower than older age group. It was revealed that prevalence of *T.gondii* infection increases with increase in age. Furthermore, it was also evident that women belonging to rural areas had more prevalence (35%) than those belonging to urban areas (6.25%) because of lower educational level, poor hygienic conditions and lower income status. Results of present study also showed that prevalence was higher (36%) in lower income group as compare to higher income group. Seropositivity was (21%) in lower income group and it was only (14%) in higher income group.

Seroprevalence was higher in women having lower weight. Seroprevalence was also higher in women belonging to rural areas and low socioeconomic status. Significant differences ( $p < 0.05$ ) were found among income group (5000-12,499) for seropositivity of Toxoplasmosis.

### **PREVALENCE OF MALARIA IN TWO TEHSILS (YAZMAN AND AHMAD PUR EAST) OF DISTRICT BAHAWALPUR**

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This study was conducted to investigate the incidence of malarial infections in human population in two Tehsils (Yazman and Ahmad Pur East) of district Bahawalpur, Punjab, Pakistan. Malarial parasites were identified in the blood slides of suspected patients of the disease from July, 2012 to June, 2013. Out of 974 suspected cases of malaria, 33 (3.38%) in Yazman and out of 1295 23 (1.78%) in Ahmad Pur East were found to be positive for malarial parasite in blood smear slides. Out of positive cases, 20 (60.60%) were identified as *Plasmodium vivax* infection, 13 (39.40%) cases with *P. falciparum* in Yazman and 13 (56.52%) were identified as *Plasmodium vivax* infection, 10 (43.48%) cases with *P. falciparum* in Ahmad Pur East. Infection with *P. vivax* in male was 63.17% and in female 57.14% whereas infection of *P. falciparum* in male was 36.83% and in female was 42.85% in Yazman and infection with *P. vivax* in male was 66.66% and in female 45.45% whereas infection of *P. falciparum* in male was 33.33% and in female was 54.55% in Ahmad Pur East. There was no case of *Plasmodium malariae* and *P. ovale* infection observed in the present study. It seems that there is no association between types of infection and age groups. Therefore, it can be concluded that the incidence of any type of infection can happen to person of any age group independently.

### **PREVALENCE OF HUMAN MALARIA IN RURAL AREAS OF DERA GHAZI KHAN DISTRICT PUNJAB, PAKISTAN**

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Malaria is one of the worldwide public health problems and imposes a major

burden on health in under developed countries of world. Half of world inhabitant is at risk of malaria with an estimated 300-500 million clinical cases and 1.5-2.7 million deaths were reported per year. The present study was conducted in the rural areas of D.G. Khan to screen the different species of malarial parasites during September 2012 to August 2013. A total 750 individuals were examined by a smear microscopy and diagnostic devices, out of which 212 (28.27%) cases were found positive. Among the total positive cases, 135 (63.68%) were infected with *Plasmodium vivax* and 67 (31.60%) were infected with *Plasmodium falciparum*. Mix infection of *Plasmodium vivax* and *Plasmodium falciparum* was found in 10 (4.71%) of the total individuals. Relationship between sex and *Plasmodium spp.* revealed a significant difference between genders. The males showed higher 148(32.89%) prevalence as compared to females 64(21.33%). Relationship between age groups and *Plasmodium spp.* showed that the parasite had highest ( $P<0.05$ ) prevalence 68(43.03%) in age group of 1-10 years and lowest 12(29.41%) in age group of > 61years.

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#### **PREVALENCE, COMPARATIVE STUDY, CLINICO-BACTERIOLOGICAL INVESTIGATION AND ANTIBIOGRAM OF SUB-CLINICAL AND CLINICAL MASTITIS IN DAIRY GOATS**

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Mastitis is common managerial problem of dairy goats in which bacteria (70%), unknown reasons (28%) and yeast and moulds (2%) are the main etiological agents. In the present study, prevalence of sub-clinical and clinical mastitis in lactating goats, comparative study of prevalence in dera din pannah, beetal, teddy, desi and non-descriptive goat's breeds of Pakistan, investigation of pathogenic bacteria of mastitis and their susceptibility to various antibiotics were tested. Milk samples were collected from ten different areas of district Muzaffargarh, Punjab and subjected to Surf Field Mastitis Test (SFMT) for screening and prevalence. The positive samples were cultured on blood agar, tryptose agar and MacConkey's agar for bacterial isolation. *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysagalactiae* and *Bacillus cereus* were isolated. After identification of organism, 10 different antibiotics were used for determination of anti-bio-gram against bacterial isolates. 235 milk samples from lactating goats of different breeds reared on grazing were tested for mastitis. Out of which 51 (21.70%) were found positive for subclinical (18.29%) and clinical mastitis (3.4%). All positive samples yielded growth of different bacteria. Enrofloxacin, Ciprofloxacin, Ampicillin, Gentamycin, Oxytetracycline, Penicillin, Amoxicillin, Norfloxacin, Chloramphenicol and

Streptomycin were tested for bacterial sensitivity. Chloramphenicol, norfloxacin, gentamicin, penicillin and ciprofloxacin were found effective *in vitro* among these.

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### VECTOR BIOLOGY OF *Aedes* AND *Culex* AND THEIR ROLE IN DISSEMINATION OF DENGUE VIRUS

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Dengue virus is now becomes a serious threat to humans. As there is, yet, no vaccine and proper treatment is available for dengue virus, so control of vector is the only option left to control dengue infection. *Aedes* mosquitoes are well known vector for this virus but some studies shows that dengue virus can also be survived in *Culex* mosquitoes. A total of 370 adult resting *Aedes* and *Culex* mosquitoes were collected from selected localities of Urban Lahore. Of collected *Aedes* (n=166) were divided into 15 pools and total collected *Culex* (n=204) were also divided into 15 pools. Of which 2 pools of *Aedes* (13.33%) and 3 pools of *Culex* (20%) were found positive for dengue virus after molecular analysis. Interestingly, only serotype-2 of dengue virus was found in all positive pools. The current study shows that dengue virus is circulating in study area. There is need of time to conduct detailed studies to establish the vectorial role of *Culex* for dengue virus.

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### COMPARATIVE STUDY OF MICROSCOPIC EXAMINATION AND RAPID DIAGNOSTIC TEST (RDT) FOR THE DETECTION AND IDENTIFICATION OF *Plasmodium* spp.

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Malaria is usually unsteady in Pakistan and its main transmission phase is post monsoon *i.e.* from August to November. Major vector are *Anopheles stephensi* and *A. culicifacies* while causative agents are *Plasmodium falciparum* and *P. vivax* that kills millions of people each year. Malaria diagnosis is a challenge to laboratories as well as research workers. Microscopic examination and rapid diagnostic tests (RDTs) are two diagnostic tools that put the largest impact on malaria control today. Present comparative study was based on two methods of rapid diagnostic tests (RDTs) and microscopic

examination in the identification of malaria. Microscopic examination based on thick and thin films preparation, thick blood film is a drop of blood on a glass slide, unfixed slide was placed in 5% Giemsa stain for 30 minutes, then washed gently in tap water and air dried which showed better sensitivity of the blood film technique and good for the detection of low levels of parasitemia but it was more difficult to find the parasites among the WBC and platelets whereas in thin blood film a drop of blood is spread across a large area of the slide, fixed in methanol and diluted Giemsa stain was used, in thin blood film the identification of different *Plasmodium* species was much easier and showed more specificity than the thick-film examination due to the presence of monolayer of RBCs. RDT is a valuable method to microscopy because it helps to diagnosis the periphery blood it was especially best practice in neglected or under developed areas where laboratory facility are often out of reach it is also helpful as a confirmatory diagnosis before the treatment. The Malaria Rapid Tests kit was found to be reliable and able to detect malaria within 15 minutes of application that was very sensitive and specific to test for all *plasmodium* species. The kit based on immunochromatographic assay for the initial detection and confirmation of *P. falciparum* (*Pf*) and *vivax* (*Pv*) antigen. Blood was taken by finger-pricking and drawn blood was poured into the glass capillary tube till it was filled then directly applied the blood from the capillary tube into the center of the cassette well and allowed for 30 seconds for the blood to be absorbed before adding the given reagent then added 5 drops of the reagent into the cassette well. Results were noted into 5 – 10 minutes. Blood of 350 patients including male and female of different age groups of four areas of Karachi were examined by microscopy and RDTs. Total 65 out of 350 tested positive to RDTs method (18.57%). In which 38 cases positive for *P.v* and 27 positive for *P.f* while 54 patients tested positive to microscopic examination (15.42%). Total positive cases were 23 and 31 with *P.f* and *P.v* respectively. All patients that were found positive to microscopy were also tested to RDTs methods. It was concluded that present investigation based on RDTs (whole blood) method is as specific as the traditional microscopy and appears more sensitive than microscopy. RDTs should be used in remote areas or it can be used as a confirmatory method before the microscopic examination to avoid the chance of misdiagnosis of different species of malaria.

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#### **SCENARIO OF DENGUE ERADICATION PROGRAMME IN TEHSIL FEROZWALA DISTRICT SHEIKHUPURA: A NEW APPROACH**

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A campaign was launched in Tehsil Ferozwala against dengue eradication after a sharp rise in dengue patients in August 2013 due to heavy rains in July/ August which increased the breeding sites of dengue mosquitoes both indoor and outdoors. Outdoor spots were generally observed in factories, junkyards, graveyards etc. Despite active

surveillance and eradication of Aedes larvae in these hot spots number of dengue patients increased alarmingly. To address the matter, the services of more entomologists were hired along with six environmental inspectors. These all were assigned different union councils. These entomologists worked with indoor teams and collected Aedes larvae and trained the indoor and outdoor teams regarding identification of larvae, their breeding sites and management of Aedes larvae. Maximum larvae were found inside the houses. On collection of Aedes larvae each team was given 1000 rupees. Along with these measures and other activities like dissemination of literature to the public, awareness raising campaigns in schools, factories, tyre shops etc. Seminars and walks were arranged for the awareness of general public/ masses. Factories and other buildings were sealed and FIR was registered against the owners who show any reluctance in adoption of dengue eradication SOPs. All these efforts resulted in significant reduction in Aedes larvae resulting sharp decline in dengue patients.

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#### **GENETIC ETIOLOGY OF CORONARY ARTERY DISEASE CONSIDERING NITRIC OXIDE SYNTHASE 3 GENE**

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The nitric oxide synthase 3 (NOS3) gene is responsible for the production of nitric oxide (NO) in the endothelial walls which maintains the vascular endothelium. It also acts as a vasoprotective agent and prevents the development of coronary artery disease. Reduced production of NO due to rs1799983 single nucleotide polymorphism in NOS3 may enhance the risk of coronary artery disease (CAD). The association of rs1799983 polymorphism with CAD was investigated in the local population of Pakistan. Study consisted of 354 individuals, out of which 182 were CAD patient and 172 were normal healthy individuals. The association was analyzed between diabetes, hypertension and smoking with CAD. Allele specific PCR based strategy was used for the detection of different genotypes of rs1799983 polymorphism. Frequency of T allele was higher as compared to G allele in our population. Strong association between rs1799983 and CAD was observed ( $p < 0.01$ ). TT genotype was found to enhance 5.717 times the risk of CAD (OR: 5.717; 95%CI 3.586 - 9.115). Hypertension was found to increase the risk of CAD by 2.572 times (OR: 2.572; 95%CI 1.625- 4.070). Smoking increased the risk of CAD development by 1.782 times (OR: 1.782; CI 1.129-2.813). On the basis of present results, it can be concluded that rs1799983 is strongly associated with CAD in our population and TT genotype of this polymorphism enhanced the risk of CAD in Pakistani population.

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### COMPARATIVE EFFICACY OF RP TEST WITH ANTI-CCP TEST IN PATIENTS OF RHEUMATOID ARTHRITIS

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Rheumatoid arthritis is an autoimmune inflammatory disease that principally affects joints. Various diagnostic techniques are in use to diagnose RA. During the present study a comparison of efficacy of RF test was done with anti-CCP for diagnostic evaluation of rheumatoid arthritis. To determine RF, latex agglutination method was used, on the other hand anti-CCP was performed using Abbot AxS-YM system for anti-CCP. The analysis showed that RF test is more efficient than anti-CCP to determine whether an individual has RA or not. In the present study RA is also found to be associated with age of the patients.

### ROLE OF DIFFERENT DEVELOPMENTAL STAGES OF Aedes IN TRANSMISSION OF DENGUE VIRUS

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*Aedes aegypti* and *Aedes albopictus* are well known vectors for dengue virus. They are more prevalent in tropical and sub tropical areas of world. Different developmental stages of these mosquitoes also play an important role in dengue transmission. Larvae act as reservoir of dengue virus during inter-epidemic period. This study was conducted in Lahore, Pakistan to determine role of developmental stages of *Aedes* in dengue infection. From March to mid of June, 2013 larvae were collected from different localities of Lahore. A total of 523 larvae were collected. Laboratory reared adult 60 *Aedes aegypti* and 45 *Aedes albopictus* were collected from NIMRT, institute of public health Lahore. Larvae and adult were divided into 47 pools and each pool contains 1-15 larvae. Molecular analysis was done to detect the dengue virus at CEMB, Lahore. Of total 40 pools of larvae, 5 were found positive for dengue. Only one pool of laboratory reared adult mosquitoes were found positive. Detection of dengue virus from field caught larvae and laboratory reared adult mosquitoes confirm the existence of vertical transmission of dengue virus in nature. A detail study should be conducted for

consecutive 2-3 years must be carried out to establish better understanding about vertical transmission of dengue virus in Pakistan. This will be proved helpful to control dengue as control of vector is important for eradication of dengue.

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**BIOACTIVITY OF MEDICINAL PLANTS EXTRACTS AGAINST DENGUE  
MOSQUITO *Aedes albopictus* (DIPTERA: CULICIDAE)**

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This study was performed to evaluate the larvicidal and pupicidal potential of the essential oils extracted from the branches and leaves of eucalyptus (*Eucalyptus globules*), neem (*Azadirachta indica*), peppermint (*Mentha spicata*), niazbow (*Ocimum basilicum*) and from rhizome of ginger (*Kaempferia galangal*) against the larval and pupal stages of *Aedes albopictus*. The essential oils were extracted by using soxhlet apparatus with petroleum ether as a solvent. The oils were evaluated against 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> instar larvae and pupae of *Ae. albopictus* using WHO protocol. The dead ones were counted after 8, 16, 24 and 48 h with different concentrations (100, 200, 300 and 400 ppm) and LC<sub>50</sub> and LT<sub>50</sub> values were also calculated. A control treatment was also run by using petroleum ether. Petroleum ether also showed a great effect on the mortality of different life stages of *Aedes* mosquitoes. Higher mortality was observed in early life stages than late life stages. Different concentrations of Eucalyptus oil showed greater mortality in different life stages than other oils. All the oils were statistically at par with each other. The ginger oil showed the least mortality in case of different life stages. All the studied factors and their interactions were found highly significant statistically.

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## 5. MICROBIOLOGY

### MPT32-pND14 BASED VACCINE AGAINST TUBERCULOSIS AND ITS EFFECTIVENESS

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DNA vaccination is the modern form of vaccine, rapidly replacing regular vaccines, with more effective and responsive. Present research designed to extract the plasmid DNA, confirm its purity and check the effectiveness of the construct *MPT32-pND14* based DNA vaccine against tuberculosis. The constructs cultured on nutrient agar. The plasmid DNA extraction performed by two methods to check purity. One with Plasmid DNA-Alkali Lysis Method and other with Endotoxin Free Plasmid DNA extraction. Plasmid DNA extraction given impure DNA confirmation as compared to Endotoxin Free Plasmid extraction. The size of extracted plasmid DNA fragments through Alkali lysis method obtained lies between 6000-8000bp. The circular DNA showed 8kbp. Size further revealed by double and single DNA digestion by *kpn1* and *pst1* restriction enzymes given 5kbp and 3kbp.

### INFLUENCE OF PROBIOTICS ON SURVIVAL AND BODY COMPOSITION OF *LABEO ROHITA* FINGERLINGS, CHALLENGED TO *PSEUDOMONAS FLUORESCENS*.

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Probiotics incorporated feed effect on survival and body composition of *L. rohita* fingerlings against *Pseudomonas fluorescens* pathogen are reported in this study. *Sphingomonas* sp. AsCh-P3 and *Bacillus subtilis* AsCh-A7 having antipathogen potential were used as probiotics. The suspension of pathogen (O.D-0.5) was injected intraperitoneally to positive control-C2 and experimental fishes. Fishes without pathogen challenge served as negative control. Both control groups were fed with sterilized fish feed. Fishes were divided into six groups *i.e.*, G1a, G1b, G1c and G2a, G2b, G2c for probiotics AsCh-P3 and AsCh-A7, respectively. Fingerlings were administered with probiotic treated feed for 45 days as; groups G1a, G2a (soon after challenge for 30 days),

G1b, G2b groups (15 days prior and 30 days after challenge) and G1c, G2c (15 days prior challenge). The survival of fishes was improved in all groups. Highest survival 80-90% was recorded in G1b and G2b as compared to 50% value of the pathogen challenged fish. *Sphingomonas* sp. AsCh-P3 supplementaion in feed resulted in significant difference in biochemical contents, except total Protein, soluble protein and RNA in G1a, G1b, G1c. The effect of *B. subtilis* AsCh-A7 was evident by significant increase in total lipids, total carbohydrates, RNA, DNA in all the experimental groups. Total proteins decreased in G2a and G2b, except G2c. The biochemical analysis and survival data demonstrated that *Sphingomonas* sp. AsCh-P3 and *Bacillus subtilis* AsCh-A7 are good probiotic candidates for *L. rohita* fresh water fish in case of the bacterial pathogen outbreaks.

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**CHARACTERIZATION AND SCREENING OF ALKALIPHILIC  
ACTINOMYCETES ISOLATED FROM A SALINE LAKE AGAINST VARIOUS  
MULTI DRUG RESISTANT BACTERIAL PATHOGENS CAUSING  
VENTILATOR ASSOCIATED PNEUMONIA**

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The rapid emergence of drug resistance among pathogenic bacteria, especially multidrug resistant bacteria, underlines the need to look for new antibiotics. In the past actinomycetes have remained the prolific sources of the largest number of secondary metabolites. Nowadays, the traditional approaches based entirely on the screening of microbes from soil have been tired and proven to be totally ineffective for the release of novel antibiotics. The present study was designed to isolate, characterize and screen the alkaliphilic actinomycetes for their antimicrobial potential against multi drug resistant bacteria causing ventilator associated pneumonia. A total of forty different actinomycetes were isolated from the water and mud samples collected from a saline Kallar Kahar lake. The taxonomic status of the isolated strains was determined by morphological, biochemical and physiological characterization as well as by 16S rRNA gene sequencing. A comprehensive biological and chemical screening strategy was adopted for the detection of potentially useful actinomycetes strains active against multi drug resistant bacteria. In biological screening, the antimicrobial activity of the crude extracts was determined using agar diffusion assay against MRSA, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter spp.*, and *Acinetobacter spp.* Additionally a cytotoxicity test was performed by brine shrimp microwell cytotoxicity assay. In chemical screening each of the crude extracts was analyzed by thin layer chromatography (TLC) using various staining reagents and high performance liquid chromatography (HPLC-UV). The current study revealed that majority of the actinomycetes strains (90%) was belonging to the genus *Streptomyces* and showing strong pH (9-11) tolerance. Among selected strains, KL<sub>14</sub>, KL<sub>21</sub>, KL<sub>29</sub>, KL<sub>32</sub>, KL<sub>37</sub>,

KL<sub>41</sub>, KL<sub>63</sub>, KL<sub>72</sub>, KL<sub>73</sub>, KL<sub>81</sub>, KL<sub>93</sub>, KL<sub>96</sub>, KL<sub>98</sub> and KL<sub>116</sub> were found to be most biologically active as they exhibited promising activity against all the test strains of multi drug resistant pathogens. In thin layer chromatographic analysis of the crude extracts, bands of different colors were visualized under short and long UV light indicating a unique pattern of different bioactive metabolites. HPLC-UV chromatograms of each crude extract of the isolates showed a number of different peaks at various retention times, which was also an indication of the presence of many valuable antimicrobial compounds in the crude extracts. Overall the study reveals that alkaliphilic actinomycetes are a promising source of commercially useful antimicrobial agents which can be exploited for further purification of unique secondary metabolites and determining their anti viral, anti parasitic, anti tumor activities as well as the structural elucidation of the bioactive compounds.

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#### PROTEASE PRODUCTION FROM MICROBES ISOLATED FROM VARIOUS CONTAMINATED SOIL SAMPLES

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Proteolytic bacteria are most important for various industries such as food and fermentation. Thus, in the current research, a total of 32 bacteria were isolated from the different waste 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 plates. Among all bacterial isolates, 8 were selected on the production maximum alkaline proteolytic activity. These 8 isolates (3 *S. aureus* (d1, f2, and H1), 4 *Bacillus spp* (b1, c1, e1, and H2), and *S. epidermidis* (C2)) were identified through various biochemical tests. Optimization of the fermentation medium for maximum protease production was carried out by using various carbon, nitrogen and substrate sources. Among these strains f2 showed maximum proteolytic activity (1240.45 U/ml) with glucose and yeast extract after the fermentation period of 24 h at 37°C whereas C2, H1 and H2 produced proteases (1089.15 U/ml, 1067.84 U/ml, and 1108.71U/ml) after 72 h of fermentation. The b1, c1, d1 and e1 produced significant amount of protease enzyme (831 U/ml, 1084 U/ml, 483 U/ml, and 946 U/ml) in the presence of glucose and peptone after the fermentation period 48 h at 37°C. Among all studied bacterial isolates, the significantly highest enzyme activity was observed by f2. It was concluded that the optimum conditions for protease production were 37°C, pH 10, glucose as carbon source and yeast extract as nitrogen source. Dehairing and blood stains were actively removed

by all tested microbes. It is well proved that the waste effluent can be used as a source for alkaline protease production which in further having many industrial applications.

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### LIPASE PRODUCTION THROUGH DIFFERENT FERMENTATION CONDITIONS

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Studies on lipase production were carried out with a bacterial strain isolated from various contaminated soil samples viz., slaughter house, dairy waste, house hold wastes, engine oil, and sludge soil by using nine production medium having different carbon, nitrogen and substrate sources with maximum pH 7 (PM1, PM2, PM3, PM4, PM5, PM6, PM7, PM8, PM9). Eight bacterial isolates viz., *S. aureus* (d1), 3 *Bacillus spp* (b1, c1, and H2), *S. epidermidis* (C2) and *E. coli* (C1) were identified through various biochemical tests. Different incubation periods (24, 48, 72, 96 and 120 h) and various temperatures were also used to produce maximum lipase enzyme. Lipase activity was recorded in both culture and cell free supernatant samples of microbes. Culture sample and cell free supernatant of H2 showed maximum lipase activity at 37°C (6.354 g/l and 8.225 g/l) in PM9 after 48 h whereas maximum activity (1.033 g/l and 4.371 g/l) at room temperature in PM9 after 96 h. It was observed that culture and cell free supernatant of *E. coli* (C1) produced maximum lipase activity of 3.79 g/l, 5.78 g/l, 4.75 g/l, and 5.37 g/l at both 37°C and room temperature in PM9 after 72 h. Cell free supernatant of c1 showed maximum lipase activity (7.96 g/l) at 37°C in PM2 after 72 h of incubation as compared to at room temperature (2.29 g/l) after 120 hrs in PM9. Similar results were recorded by *S. epidermidis* (C2) as 8.23 g/l in PM1 after 72 h and 3.01 g/l after 120 h of incubation in PM2 at room temperature. Interestingly Culture and cell free supernatant of b1 illustrated maximum lipase activity (4.72 g/l and 8.77 g/l) after 48 h of incubation at 37°C using PM7 whereas 10.240 g/l and 14.18 g/l was recorded after 96 h in PM9. On the other hand almost equal amount of lipase activity was produced by both culture and cell free supernatant of *S. aureus* (d1) at both 37°C (PM2 after 96 h) and room temperature (PM1 after 120 h) such as 4.026 g/l, 4.77 g/l, 5.60 g/l, and 5.69 g/l, respectively. Removal of oil stains from fabrics was also analyzed and observed that cell free supernatant had significant activity. It was concluded that tested microbes produced extracellular lipase enzyme and could be used to hydrolyze fats and oils on industrial scale.

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### **ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF *BACILLUS THURINGENSIS* FROM THE SOIL SAMPLES OF HAZARA REGION**

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*Bacillus thuringiensis* (*Bt*) is a gram positive, soil dwelling bacterium possessing insecticidal characteristics. The insecticidal activity is due to its ability to synthesize large amount of crystal proteins called delta endotoxins or crystal (Cry) proteins and it is the most widely used biocontrol agent. Employed as a biocontrol agent for many decades against lepidopteron insects, *Bt* formulations are known to have a narrow host spectrum, harmless to humans, mammals and non-target insects. The aim of the present study was to isolate and characterize *Bt* strains from the local agricultural soil of Hazara division. *Bt* was isolated from 55 soil samples collected from diverse crop fields of Mansehra, Haripur, Havellian, Abbotabad and Batagram districts by using the acetate selection protocol of Travers *et al.* (1987) with some modifications and were subjected to further morphological and biochemical characterization. Sixty seven isolates were presumptively confirmed as *Bt*. All the isolates were gram positive. All of them were able to hydrolyze starch, casein, decompose tyrosine, indole negative, motile in semi agar medium and showed resistance to 7% NaCl and 0.001% lysozyme. Among these *Bt* isolates 39% were isolated from Haripur soil samples, 17.2% from Abottabad, 25% from Havellian, 15.6% Mansehra and 3.2% were isolated from Batagram soil samples.

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### **ENHANCED RICE PRODUCTIVITY BY INOCULATION WITH RHIZOBACTERIA**

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The present study focuses on the beneficial effect of an indigenous rhizobacteria *Serratia marcescens* isolated from the roots of rice. Crude bioactive compound was extracted from *S. marcescens* by phase separation technique. Over production of bioactive compound in wild-type strain was achieved by media manipulation and random mutagenesis. The high-yielding derivative mutant was analyzed for genetic diversity by amplification of the chromosomal DNA followed by agarose gel electrophoresis. The extracted compound was tested *in vitro* for its potential antimicrobial activity against fungal pathogen of rice by dual culture assay and up to 65% fungal growth inhibition was recorded. The growth promoting effect of *S. marcescens* was evaluated *in vivo* by applying bacterial inoculant on rice seedlings of different rice genotypes at the transplanting time. Genotypic variations were observed in growth and yield response to

bacterial inoculum. The maximum beneficial effect of inoculation with *S. marcescens* was observed in growth and yield parameters of Basmati-370 and a 39% increase was recorded for grain yield (g/plant) as compared with the control. Results indicated the biosynthetic capability of indigenous bacterial strain and its potential application as a biological inoculant for enhanced rice productivity.

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**BENEFICIAL ROLE OF AUXIN PRODUCING *BACILLUS* STRAINS IN  
CHANGING THE ROOT ARCHITECTURE OF *ARABIDOPSIS THALIANA* AND  
GROWTH PROMOTION OF *ZEA MAYS* PLANT**

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Rhizospheric bacteria involve themselves by playing an imperious role in development and growth of plant by diverse mechanisms, among which auxin production is well studied for its effect in changing the plant root morphogenesis. Three strains PNS-2, PNS-12 and PNS-13 were isolated from rhizospheric region of different plant roots, characterized biochemically and further identified by I6S rRNA gene sequencing. These isolates were screened for auxin production by Salkowski's method and further confirmed by high performance liquid chromatography coupled with mass spectrophotometry. Effect of these auxin producing strains on root architecture was evaluated by using model plant *Arabidopsis thaliana* by designing a sand system to observe the root architecture up to tertiary root level which was not possible in soil system. Auxin producing rhizobacteria caused the significant ( $P < 0.05$ ) increase in the primary root length of *Arabidopsis thaliana* and changed the pattern of secondary roots by increasing their numbers. Efficacy of these strains was further checked on *Zea mays* growth under laboratory and field conditions. The strains were found to cause the significant ( $P < 0.05$ ) improvement the growth parameters of the maize seedlings as compared to the non-inoculated ones under laboratory conditions. Similarly, bacterial inoculation caused a significant ( $P < 0.05$ ) increase in the yield parameters of the plant as compared to the non-inoculated ones under natural condition. In the present study the sand system was used as a model system close to the natural environment to check the root architecture of the plants, changed by bacterial inoculation, without damaging the delicate root as in soil system. Moreover, our results revealed the fact that field experiments may helpful in selecting the best candidates as PGPR for future purposes along with *in vitro* screening procedures.

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### ASSESSMENT OF METAL' INDUCED FUNCTIONAL STRESS IN SULPHATE-REDUCING THERMOPHILES

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Various toxic metals have been known to inhibit different activities of sulphate reducing bacteria (SRB) at different concentrations. The present study reports functional responses of two thermophilic SRB species (*Desulfotomaculum reducens*-HA1 and *Desulfotomaculum hydrothermale*-HA2) isolated from a hot water spring in Chakwal, Pakistan. Bacterial activity was assessed in terms of sulphate reduction and metal precipitation employing four concentrations (1 ppm, 5 ppm, 10 ppm and 15 ppm) of three dissolved metals (Cu, Cr and Ni) independently. Both sulfidogenic bacterial species showed results in a very narrow range of fluctuations. In general, bioprecipitation and sulphate reduction were pronounced at lower concentrations (1 and 5 ppm) and get inhibited at higher concentrations (10 and 15 ppm). The order of precipitation and sulphate reduction for the subject metals was Ni<sup>+</sup>, Cr<sup>+</sup>, Cu<sup>+</sup>. The findings of this study will be helpful in developing economical and environmental friendly bioremediation process(es) tending to operate at extreme conditions around the concentrations in indicated suitable metals' effluents.

### *PSEUDOMONAS AEUROGINOSA* AND *STREPTOCOCCUS HEMOLYTICUS* IN THE LARVA *PRZHEVALSKIANA SILENUS (BRAUER)*, A UNIQUE COMBINATION; FACULTATIVE OR COMMENSALS

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*Pseudomonas aeuroginosa* and *Streptococcus hemolyticus* were isolated and observed inside a hundred larvae of *P. silenus brauer* of Rohila goat breed of Dera Ghazi Khan in December, 1997. The study was unique in its nature to explore the facultative or commensal bacterial help of larva of goat warble during its L2 migration or its L3 sojourn.

### COMPARATIVE ASSESSMENT OF BIODIESEL PRODUCTION FROM MICROALGAE (CHLOROPHYTES AND CYANOPHYTES)

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One of the main global problem as well as in Pakistan is the continuous depletion of fossil fuels and it's declined production. Researchers in the field of chemical and biotechnology are working on the alternates of fossil fuels. Cry of the day is to adopt new and safest methods for the distribution and storage of energy. Biofuel is referred as energy obtained from biomass. Different sites of northern areas (Naran, Balakot, Jareed) and University of the Punjab, Lahore were selected for sample collection. About 62 different microalgal strains (from Chlorophyta and Cyanophyta) were isolated, cultured and identified morphologically and genetically. Screening of hyper lipid accumulating isolates was performed by Sudan black staining, by which MFF 19, MFF 12, MFF 10 and MFF 6 showed both intracellular and secretory lipids while MFF 3, MFF 8, MFF 10 and MFF 13 contained just intracellular lipids. Total lipids were extracted from the three weeks incubated culture and then converted to biodiesel through transesterification. Five selected isolates were grown in field and no significant difference was observed in field and lab conditions, which is a promising result for diesel production from mass cultivated microalgae. Consortia showed best results as compared to their representative isolates. Unicellular isolates showed higher percentage yield of biodiesel. FTIR analysis was also performed to find out the presence of functional groups present in biodiesel. Algal oil and diesel production was significantly higher in Phormidium, Oscillatoria, Leptolyngbya and Microcoleus (filamentous isolates), Gloeobacter, Cyanotheca, Synechococcus and Chlamydomonas (unicellular isolates). Thus microalgae can be an efficient source of various biofuels in future. Cyanobacteria are as efficient as chlorophyta for biodiesel production and both can be potential source for compensating the deficiency of diesel.

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### ISOLATION AND LOW-COST CULTIVATION OF XANTHAN GUM YIELDING BACTERIA FROM DIFFERENT SOILS

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Xanthan gum is a natural biopolymer having important industrial applications such as food additives and in cosmetics. It is commonly known as heteropolysaccharide which is mainly produced by *Xanthomonas campestris*. In the present study bacteria were isolated on YDC agar medium at room temperature (28±1°C). Two categories of the



bacterial isolates' colonies differed from each other in having bright or light yellow appearance. Cultivation of the bacterial isolates in certain agro-industrial wastes is likely to provide xanthan gum at low-cost.

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**ALBORIXIN: THE POLYETHER ANTIBIOTIC, PURIFICATION AND  
STRUCTURE ELUCIDATION FROM AN INDIGENOUS ISOLATE  
*STREPTOMYCES PULCHER* CRF17**

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In our search for antimicrobial compounds from *Streptomyces* spp. and rare actinomycetes of different ecological niches in Pakistan, an isolate *Streptomyces* sp. CRF17 isolated from a saline soil was investigated for its antimicrobial activity against different bacterial, fungal and algal test organisms. The biological screening revealed that the isolate exhibit potent antimicrobial activity against gram positive and gram negative bacterial test organisms along with minor antifungal and anti algal activity. The scale up fermentation of the isolate *Streptomyces* sp. CRF17 as 20 liter lab fermenter (Biostat E) and extraction of the culture broth by ethyl acetate, acetone and methanol yielded 0.71 g crude extract. The subsequent isolation and purifications of the crude extract using several chromatographic techniques yielded one pure compound. The purified compound was identified by mass spectrometry (ESI and HRESI-MS) and by NMR analysis (<sup>1</sup>H and <sup>13</sup>C NMR) as alborixin (**1**), which was further, confirmed by comparison with the literature data. The morphological, biochemical and physiological characterization suggested that isolate CRF17 belongs to the genus *Streptomyces*. Further a partial 16S rRNA gene sequence 1420 bp (gene bank accession number: EU294134) from the isolate CRF17 was determined and found to have high similarity 98 % with *Streptomyces pulcher*.

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**OPTIMIZATION OF CELLULOLYTIC POTENTIAL OF BACTERIA  
ISOLATED FROM FISH GUT**

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The present study was undertaken to screen cellulolytic potential of three bacteria isolated from the gut of freshwater fish, *Catla catla*. These isolates were identified through 16S rDNA gene sequencing as *Bacillus amyloliquefaciens*, *Enterobacter aerogenes* and *Bacillus vallismortis*. Optimization for maximum cellulase production was carried out. The culture conditions like pH, temperature and inoculum size were

optimized by using cellulose selective medium. The bacterial species *B. amyloliquefaciens* liked neutral pH while the two other species gave maximum yield at pH 5. *B. amyloliquefaciens* showed maximum cellulase production at 37°C, while the optimum temperature for cellulase production for the *E. aerogenes* and *B. vallismortis* was 45°C. When different inocula sizes were attempted the bacterial specie *B. amyloliquefaciens*, *E. aerogenes* and *B. vallismortis* yielded best enzyme production when the cultures were inoculated with 2%, 10% and 5% inocula, respectively. The *E. aerogenes* yielded upto 293 enzyme units (U/ml) which was best in comparison with 248 U/ml of *B. amyloliquefaciens* and 195 U/ml of *B. vallismortis*. The high activity as well as thermostability of the bacterial cellulases may find industrial and biotechnological applications. The autochthonous bacterial species are promising for the probiotic administration to enhance fish growth and promote aquaculture development.

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**MOLECULAR CHARACTERIZATION OF BACTERIAL STRAINS ISOLATED FROM FENITROTHION AND DIAMETHIOATE CONTAMINATED SOIL OF DISTRICT GUJRAT PAKISTAN**

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Pesticides, the substances used for pest control have been used extensively. These damp on crops, soil and also contaminate ground water. Therefore, after pesticides have been done their work these must be cleaned out from the environment so that their toxic metabolites must be degraded. Most commonly used pesticides are the organophosphates. Biodegradation is one of the promising methods to eliminate pesticides from the environment. Many bacterial species participate in this process, among which *Arthrobacter*, *Burkholderia*, *Plesiomonas*, *Agrobacterium*, *Pseudomonas* and *Bacillus*, are included. In the present study two bacterial strains APDB9 and APDB10 isolated, were closely related to *Bacillus megaterium* and *Bacillus thuringiensis*, respectively. Considerable concentration of pesticide residues Fenitrothione and Diamethioate were detected by HPLC in these agricultural fields' soil samples. All bacterial strains contained a plasmid of 21 kb size, which suggests the involvement of this plasmid to impart pesticide tolerance to these bacterial strains. Gene specific primers were used to detect, amplify and sequence the organophosphate degrading (*opd*) gene, but no amplification was obtained, which suggest that these bacterial strains contained different nucleotide sequence than already reported *opd* genes. The information may help to characterize and investigate these new types of *opd* genes, so that they may be utilized in pesticides biodegradation.

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### **ISOLATION AND CHARACTERIZATION OF BACTERIAL FAUNA ISOLATED FROM DIFFERENT TYPES OF WOUND FROM DIFFERENT HOSPITALS OF LAHORE**

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In the present work, the incidence of bacterial population in wound samples taken from Services, Mayo and Jinnah Hospitals was studied by swabbing samples on LB Agar Medium Plates. Samples were collected from pus, diabetic foot, burn, bed sores and post operative wounds. Twenty four bacterial isolates were selected from the samples for identification. From wound samples of Services Hospital the bacterial strains isolated were as *Staphylococcus aureus*, *Streptococcus pyogene*, *Pseudomonas aeruginosa* and *Neisseria sp.* while *Klebsiella sp.*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* were isolated from wound samples of Jinnah Hospital. Mayo Hospital's wound samples were mostly of patients with 80%, 25% and 20% burns. The bacterial strains identified from Mayo Hospital samples were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Streptococcus pyogene*, *Escherichia coli* and *Klebsiella sp.* Antibiotic resistance of these strains was observed. Optimum growth conditions including temperature and pH were also studied. For plasmid isolation from antibiotic resistant bacterial strains miniprep method was used and after gel electroporation no plasmid was isolated from these bacterial strains.

### **VARIATIONS IN MICROBIAL COMMUNITY OF *HELIANTHUS ANNUUS* ACROSS THE GROWTH SEASON**

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Phytosphere is a unique and diverse microbial habitat which comprises of three territories *i.e.* phyllosphere, endosphere and rhizosphere. Phyllosphere is an unreceptive habitat for microbes due to swift alterations in surrounding environment. Endosphere is a stable environment while rhizosphere has a fundamental significance to all life forms. Overall, phytosphere niche is of great economic and environmental significance. It was considered that phyllosphere would show more microbial diversity and variations than rhizosphere as it is more exposed to the environmental variations, provided basis for this research. In addition, the impact of plant developmental stages and physiological changes on the diversity of microbes and variation in bacterial community was tried to reveal.

Culture dependent conventional method was used to exploit the culturable bacterial density. For this purpose three different media: N-Agar, TSA media and 523 Media were used to isolate maximum culturable bacterial density. The trend of cellulose and pectin degradation activity was revealed by using cellulose congo-red agar media and pectin containing minimal media. Throughout the growth season a noticeable change in community composition was observed instead of diversity. The diversity of phyllosphere and rhizosphere was almost similar at genus level. So the selection of bacterial diversity was taken as the function of spatial and temporal abiotic factors. Pectin degradation activity and the community size of phyllobacteria were considerably higher than rhizobacteria and overall more consistent community size was noticed in leaf histoplane. Many of the leaf endophytes were considered to be residing within tissues as they were able to degrade pectin, the promising component of plant cell wall. Strong association of Gram positive bacteria was seen with *Helianthus annuus*. Overall bacterial genera inhabited the plant surfaces were *Pseudomonas* sp., *Aeromonas* sp., *Bacillus* sp., *Micrococcus* sp., *Enterococcus* sp. and *Staphylococcus* sp.

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#### EVALUATION OF HEAVY METALS' BIOREMEDIATION POTENTIAL OF BACTERIAL SPECIES ISOLATED FROM RIVERINE FISH GUT

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Ever accelerating industrialization is exclusive perpetrator in polluting this sphere. Industrial effluents are rich of recalcitrant and detrimental pollutants. Bioremediation is an ecofriendly approach for the removal of the heavy metals from environment. In the present study, five enteric bacterial species isolated from fish gut content of River ravi and identified by 16s rDNA identification process were assessed for their metal resistance potential in 2% aqueous extract of watermelon peels as a low cost sustenance source under optimized conditions. MIC for the *Obesumbacterium proteus*, *Raoultella ornithinolytica*, *Aeromonas bestiarum*, *Bacillus megaterium* and *Buttiauxella ferragutiae* ranged from 500 to 750, 500 to 750, 550 to 650 and 450 to 650 µg/ml respectively for Cu (copper), Ni (nickel), Mn (manganese) and Cr (chromium). Cultivation of these bacterial species in 2% watermelon aqueous extracts in the presence of 300µg/ml of Cu and Cr for *Obesumbacterium proteus* and *Buttiauxella ferragutiae* resulted into 10.43 and 7.8 % removal. While 450, 150 and 350 µg/ml of Ni, Mn and Cr for *Raoultella ornithinolytica*, *Aeromonas bestiarum* and *Bacillus megaterium* resulted into 14.81, 11.72 and 11.08 % removal of the metal from the medium, as assessed through atomic absorption spectrophotometer after 48hrs of incubation at the pre-determined growth optima. The results indicate that these bacterial species can be exploited as good candidates for economized bioremediation of heavy metals contaminated environments.

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### MUTAGENESIS OF *BACILLUS SUBTILIS* THROUGH EMS FOR ENHANCED PRODUCTION OF TRYPTOPHAN

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The present study refer to the recuperation of *Bacillus subtilis* strain WA1 for tryptophan production in 250 ml Erlenmeyer flasks. *Bacillus subtilis* isolated from soil samples collected from sugar factories area in pattoki and Lahore . Bacterial strain grown on different fermentation media based on glucose urea and molasses to obtain maximum yield of tryptophan . Chemical mutation using ethyl methane sulfonate ( EMS 50-300 u/ml ) was undertaken for 10-30 min. Tryptophan produced by bacillus subtilis WA1 and its mutant EMSA1 observed quantitatively by acidic ninhydrin method at optical density was recorded by spectrophotometer at 370 nm for tryptophan. Maximum amount of tryptophan produced by WA1 was 8.8g/l , in MF2 which is enhanced by EMSA1 was 13.7g/l during different incubation period under optimum condition .The aim of this work is to develop a potent high yielding , feedback insensitive mutant strain and optimization of its medium pH for maximum production of tryptophan .

### ISOLATION, CHARACTERIZATION AND PARTIAL PURIFICATION OF LIPASE FROM *BACILLUS* SPP.

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The present study describes the isolation, identification and screening of Bacterial Strains for lipase production. The strains were initially selected qualitatively on Tributyrin agar plate. The most potent strains NL-37, NL-39 and NL-40 producing  $26.30 \pm 0.20$ ,  $25.10 \pm 0.20$  and  $24.4 \pm 0.40$  of lipase respectively were selected. The strains were then identified on the basis of standard morphological, biochemical test and by 16S rDNA amplification and sequencing, assigned the code NL (Nutrition Lab) NL-37 for *Bacillus Cereus*, NL-39 for *Bacillus Subtilis* and NL-40 for *Bacillus Amyloliqueficans*. Various waste oils, fruit peels and agro industrial wastes were examined for lipase production. In the presence of 5% bagasse with 5% mustard oil cake the maximum lipolytic activity was 40.50, 16.23 and 33.33 U/ml for NL-37, NL-39 and NL-40 respectively were observed. NL-37 Showed maximum lipase activity at temperature 40°C, pH 8 with 5% inoculum after 72 hours. NL-39 showed maximum lipase production at 45°C of temperature, at pH 7 with 5% inoculum. Whereas optimum conditions for lipase production by NL-40 were at 40°C temperature, pH 7 with 4% inoculums. All the present strain showed maximum production of lipase when media is supplement with

yeast extract and sucrose.  $\text{Ca}^{+2}$  showed stimulatory however  $\text{Ag}^{+2}$  and SDS expressed inhibitory effect on the production of extracellular lipases by *Bacillus* Spp. The effect of pH, temperature, detergent and metal ions showed stability of Lipases at wide range. The enzyme was subjected to purification by ammonium sulphate precipitation for salting out proteins. Desalted enzyme was subjected to DEAE – Cellulose column for ion-exchange, chromatography. Lipase from NL-37 showed 56 kDa whereas NL-39 and NL-40 showed 41 kDa and 45 kDa of molecular weight on SDS-PAGE. Biodetergent and bioremediatory properties of lipase were showed it is best for commercial uses.

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**SENSITIVITY PROFILE OF TANNERY WASTE BACTERIA AGAINST THE  
EXTRACTS OF GINGER (*ZINGIBER OFFICINALIS*) AND TAMARIND  
(*TAMARIDICUS INDICA*)**

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In the present study the antimicrobial activity of extracts of Ginger (*Zingiber officinalis*) and Tamarind (*Tamaridicus indica*) in organic solvents benzene, chloroform, ether and toluene were tested against tannery waste bacteria namely *Aerococcus viridians*, *Bacillus* sp., *Corynebacterium bovis*, *Staphylococcus epidermidis* and *Streptococcus mutans* and test organisms *Escherichia coli* and *Staphylococcus aureus* using agar dilution method. Ether ginger extract (0.1 mg/ml) showed inhibition in *A. viridians*, *Bacillus* sp. *C. bovis*, *S. mutans* whereas other extracts of ginger showed no inhibition in the growth of bacterial strains tested. Chloroform tamarind extract (0.1mg/ml) was effective against *A. viridians* *C. bovis* and *Staphylococcus aureus* while benzene tamarind extract (.01mg/ml) inhibit the growth of *E.coli* only. The ginger extracts are more effective than tamarind as antimicrobial agent. The most effective inhibition was produced by ether ginger extract (1mg/ml) against *A. viridians* in 10mintues, whereas benzene tamarind extract (1mg/ml) affected in 20-60 minutes, ether ginger extract (1mg/ml) also inhibited the growth of *Bacillus* sp. and *S. mutans* in 1440 minutes. No inhibition was produced by these extracts in *C. bovis*, *E.coli* and *S. aureus*. *S. aureus* and *Bacillus* sp. were resistant to all antibiotics (0.1mg/ml) *E.coli* was sensitive to Streptomycin; *A. viridians* was sensitive to Ampicillin Ceftizoxime sodium, Cephadrine and Streptomycin. *S. epidermidis* was sensitive to all anti biotics and *S. mutans* was sensitive to Penicillin benzyl and Streptomycin. Some of bacterial; isolated are sensitive to antibiotics but are least sensitive to tamarind and slightly sensitive ginger extract so to a very low level these extracts can be used as an alternative method for inhibiting the growth of these bacteria.

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**MOLECULAR STUDIES ON SYNERGISM OF HETERODIMERIC  $\beta$ -  
GALACTOSIDASE FROM LOCALLY ISOLATED *LACTOBACILLUS*  
*ACIDOPHILUS* MR-24**

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The aim of the present study was to isolate and characterize the  $\beta$ -galactosidase from bacteria isolated from yogurt, fermented milk, cheese and effluents of dairy industries of Lahore, Pakistan. A total of 50 bacteria were selected and were identified as Lactobacilli by Gram stain and standard bacteriological and biochemical methods. Their ability to hydrolyze 5-bromo-4-chloro-3-indolyl  $\beta$ -D-galactopyranoside (X-Gal) was also determined. For quantitative screening 15 Lactobacilli colonies that produced dark green blue color on X-Gal plates with  $\beta$ -galactosidase enzyme were selected for further studies. The qualitative screening was also done to screen the best producer of  $\beta$ -galactosidase. Highest total activity (905.15 U/l) of  $\beta$ -galactosidase enzyme was observed in one of the strain isolated from fermented milk and this bacterium was identified as *Lactobacillus acidophilus* MR-24 after ribotyping and 16SrRNA gene sequence analysis. *Lactobacillus acidophilus* MR-24 was found capable to produce glucose from substrate lactose. The culture conditions were optimized for the production of  $\beta$ -galactosidase in shake flask using wild type *Lactobacillus acidophilus* MR-24. It was found that maximum production of enzyme was obtained 48 hours of incubation time at the temperature of 37°C and pH 7. Among various carbon and nitrogen sources and raw natural sources, sucrose and beef extract and wheat flour were increased the enzyme production. The native  $\beta$ -galactosidase *Lactobacillus acidophilus* MR-24 was purified by using Sephadex-G-75 column. The enzyme assays of purified  $\beta$ -galactosidase showed 10 folds increase in activity. For the amplification of full length  $\beta$ -galactosidase gene 2.8kb lacLM, lacL (1887bp) and lacM (951) from *Lactobacillus acidophilus* MR-24, the DNA was isolated and PCR were performed. The  $\beta$ -galactosidase genes were ligated in cloning vector pTZ57R. Sequencing and sequence analysis revealed that  $\beta$ -galactosidase gene consist of two overlapping regions lacL and lacM. The lacL gene consist of 1887 bp which encodes the larger  $\beta$ -galactosidase protein subunit 628 amino acid with molecular mass 73230.5 Da while lacM gene was 951bp smaller subunit 316 amino acids encode polypeptides with molecular mass of 35817.4 Da. The two coding regions were cloned separately and together in *E. coli* using an expression system based on the T7 RNA polymerase promoter. The activity of both polypeptides was determined after expression. It was found that expression of lacL and lacM proteins alone were not yielded the active proteins but coexpression of both lacL and lacM resulted into active protein. The enzyme assays of expressed purified  $\beta$ -galactosidase showed 30 folds increase in activity when compared with crude native  $\beta$ -galactosidase. Purified  $\beta$ -galactosidase was characterized regarding pH, temperature, inhibitors and effect of metal ions. The purified native and expressed  $\beta$ -galactosidase was stable at pH 7 and with little activity outside pH 3.0 and pH 9.0. The native and expressed purified  $\beta$ -galactosidase showed highest activity at the

temperature 60°C and 45°C respectively but lost their 40% activity at 90°C. Native and expressed purified  $\beta$ -galactosidase was not inhibited by EDTA but strongly inhibited by Phenylmethylsulfonyl fluoride (PMSF) and p-chloromercuribenzoic acid while Iodoacetamide had very little effect and di-thiotheritol was found to enhance the enzyme activity. Metal ion studies showed that  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  ions increased the expressed purified enzyme activity other have no significant effect on enzyme activity. The expressed purified  $\beta$ -galactosidase cloned from *Lactobacillus acidophilus* MR-24 might be a useful source as probiotic to dairy products, could help the lactose intolerant people.

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**EFFECTS OF DIFFERENT STRAINS OF DIETARY PROBIOTICS ON  
DIGESTIVE ENZYMES OF JUVENILE GRASS CARP (*CTENOPHARYNGODON  
IDELLA*)**

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The study was conducted to evaluate the effects of three different strains of dietary probiotics (*Geotrichum candidum*, *Saccharomyces cerevisiae*, *Enterococcus faecium*) on digestive enzymes activities of juvenile grass carp (*Ctenopharyngodon idella*). Five 35% protein experimental diets, T1, T2, T3 and T4 supplemented with *G. candidum*, *S. cerevisiae*, *E. faecium* and combination of three strains respectively at a concentration of  $10^{09}$  CFU  $\text{kg}^{-1}$  and basal diet (C) without supplementation were prepared. An experiment was conducted in triplicate while on the basis of diets aquaria were divided into 5 experimental groups. The first group of fish offered basal diet while others experimental groups were fed with their respective diet twice a day at the rate of 3-4% body weight for 90 days. Results indicated that the supplementation of probiotics *G. candidum* alone or in combination with *S. cerevisiae* and *E. faecium* in the feed of grass carp significantly improved intestinal amylase and cellulase activities while showed no significant effect on protease activity. These results suggest the possible application of *G. candidum* for the enhance production of grass carp.

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**DIAGNOSTIC EFFECT OF VARIOUS ANTIBIOTICS' ASSAY ON DOMESTIC  
BIRDS**

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The research was conducted to isolate and identify the microflora from the captured birds and to evaluate the antibiotic sensitivity of the bacterial isolates. A total of



120 samples were collected during March 2011 to September 2012. The samples were cultured on different bacteriological culture media and the microbes were identified following the cultured and biochemical properties. The bacteria isolated from the domesticated birds included *Escherichia coli*, *Salmonella typhi*, *Salmonella enteritidis*, *Shigella* spp, *Staphylococcus* spp, *Proteus vulgaris*, *Pseudomonas* spp, *Klebsiella* spp. and some unidentified bacteria. The results of antibiotic sensitivity tests revealed that tetracycline is the most effective against *E. coli*, and chloramphenicol comes second. Ciprofloxacin is susceptible to *E. coli*, and *Salmonella*. Gentamicin is sensitive to *Salmonella* and resistant to *Pseudomonas* while tetracycline is resistant to *E. coli*, but sensitive to *Vibrio cholera*. *E. coli* is resistant to chloramphenicol but *Pseudomonas* and *Vibrio cholera* are sensitive to it. The antibiotics assay to treat the bacterial infections of birds showed various clinical signs like diarrhea, fever, scratching at different body parts, loss of body weight, swelling on feet and reduced food and water intake. The histological analysis revealed some signs of infection in liver and kidney samples. This clearly indicates that the application of antibiotics mainly effect the liver and kidneys of the birds. However, the most adverse effect on liver was seen in case of ciprofloxacin, while amoxicillin was injurious for kidneys. The tubular structure of kidney cells was changed in case of amoxicillin treatment in some birds.

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#### DIAGNOSTIC ANALYSIS OF WATERFOWL BASED HOST-MICROBE RELATIONSHIP

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The research was conducted to diagnostically analyze the waterfowl on the basis of host -microbe relationship. A some of 300 samples (150 buccal swabs and 150 cloacal swabs) were collected aseptically from 150 waterfowl from river Indus at Taunsa. All of these samples were serially diluted and placed on PCA. Thereafter, colonies appeared on PCA. By adopting the quadrant streaking technique, the colonies were cultured and sub-cultured on differential or selective media *i.e.* MacConkey Agar, SS agar, TCBS Agar. The morphology of bacterial isolates was further identified by Gram's Staining. All of the bacterial isolates were found Gram-negative. A total of five bacterial genera were isolated *i.e.* *E.coli* 43.33%, *Shigella* spp. 42.33%, *Vibrio* spp. 34.66%, *Salmonella* spp.14.33% and *Klebsiella* spp.5.66%. *E.coli* was the most frequent isolate followed by *Shigella* spp. Overall 44.89% bacteria were isolated from buccal swabs and 55.10% from cloacal swabs. The four bacterial isolates *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Klebsiella* were isolated in greater number from cloacal swabs except *E.coli* which were most frequently isolated from buccal swabs 53.33% than cloacal swabs 33.33%. Some 110 bacterial isolates were tested for susceptibility to eight different antibiotics Octadisc (G-XX1-minus) using the ASS agar media and the Disk Diffusion Method. Zone of inhibition were measured according to the reference table. *E.coli*, *Klebsiella*, *Salmonella*

and *Vibrio* spp. were 100% sensitive to Gentamicin and *Shigella* spp. were 92% sensitive to GEN. Overall 83.63% bacteria were sensitive to Chloramphenicol. Individually the sensitivity to C was *Shigella* 100%, *E. coli* 88%, *Salmonella* spp. 83%, *Vibrio* spp. 79% and *Klebsiella* 50%. Overall sensitivity to Ciprofloxacin and Ceftriaxone was same 69% for *E. coli*. *Klebsiella* were 82% and 83% sensitive to Ciprofloxacin and Ceftriaxone respectively, *Salmonella* spp., *Shigella* spp. and *E. coli* were found 83%, 75% and 70% sensitive to Ceftriaxone respectively. 83% *Klebsiella*, 82% *E. coli*, 67% *Salmonella*, 67% *Vibrio* and 58% *Shigella* were sensitive to Cefuroxime. Overall 49% bacterial isolates were sensitive to Ampicillin. *Klebsiella* 67%, *E. coli* 59%, *Vibrio* spp. 57% *Shigella* spp. 42% and *Salmonella* 0% sensitive to Ampicillin. Overall 45.4% bacteria were sensitive to Co-Trimoxazole. Tetracycline was least sensitive, it was only 10.9% sensitive to bacterial isolates. *Salmonella* spp. were 0% sensitive to Tetracycline. Overall 54.54% bacterial isolates were resistant to Co-Trimoxazole. *Salmonella* spp. 67%, *Vibrio* spp. 64%, *Shigella* 58%, *E. coli* 47% and *Klebsiella* were 33% resistant to Co-Trimoxazole. Overall 40% bacterial isolates were resistant to Tetracycline, *Salmonella* spp. 67%, *E. coli* 59%, were resistant to Tetracycline. Overall 32.72% bacterial isolates were resistant to Ampicillin. *Salmonella* spp. were 83% resistant to Ampicillin. The Resistance level of bacterial isolates was 10.9% to Cefuroxime, 7.27% to Chloramphenicol, 7.27% to Ciprofloxacin, 7.27% to Ceftriaxone and only 1.818% to Gentamicin. The sensitivity level of bacterial isolates was significantly different from resistant ones  $P < 0.05$ .

#### SELENIUM BIOFORTIFICATION OF WHEAT: POTENTIAL APPLICATIONS OF MICROBES IN BIOFORTIFICATION AND PHYTOREMEDIATION

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Selenium (Se) is an essential trace element for humans, livestock and other animals. Most of dietary Se is derived from crops. To develop a Se biofortification strategy for wheat, the effect of selenium-tolerant bacterial strain YAM2, showed 99% homology with *Bacillus pichinotyi*, was tested for its capacity to enhance growth, Se accumulation, and acid phosphatase activity in wheat, growing in Se-fertilized soil. YAM2 inoculation as well as Se-treatment enhanced wheat growth. Both inoculated as well as Se-treated-inoculated plants showed significantly higher dry weight, shoot length and spike length compared to un-inoculated control plants. Se-treated plants showed higher shoot length (5%), dry weight (38%) and spike length compared to control plants without Se. In the presence of Se, bacterial inoculation significantly enhanced Se, S, Fe and Ca concentration in wheat kernels and stems compared to uninoculated plants.

Inoculated Se-treated plants showed a significant increase in acid phosphatase activity also, which may have contributed to the enhanced growth. Inoculation with Se-tolerant strain YAM2 is a promising biofortification strategy for wheat and potentially for other crops and Se fertilization can effectively fulfill adequate daily Se requirements for humans and livestock.

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**ISOLATION, SCREENING AND IMPROVEMENT OF LOCALLY ISOLATED  
WILD TYPE BACTERIA FOR LYSINE PRODUCTION UTILIZING AGRO-  
INDUSTRIAL WASTE BY PRODUCTS**

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*NIAB, Faisalabd*

Research programme regarding the up-gradation of agro-industrial waste for value-added products, amino acids particularly lysine, was done. Nearly 50 bacterial isolates in different media were exploited. Majority of the wild type strains produced alanine, aspartic acid and glutamic acid upto an amount of 4.3, 2.5 and 1.8g/l, respectively, within 48 to 72 hr of fermentation at  $29\pm 1^{\circ}\text{C}$  and 150 rpm. In addition, some other amino acids, such as leu, Iso, Val, His etc. were also produced but just in traces. On the bases of their amino acid production potential, the isolates were mutated. More than 70 mutants were derived and tested for their ability to produce lysine in the fermentation broth. Minimum inhibitory concentration (MIC) for NTG and AEC was found to be 50 and 625 $\mu\text{g/ml}$ , respectively. Among them, 3 mutants found AEC-resistant, were tested for their ability to produce lysine in different molasses media. Initially, the production of a wild type isolate, NIAB KN-98, was increased from negligible to a level of >4.5g/l through mutation at  $29\pm 1^{\circ}\text{C}$  within 72 hours of fermentation.

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**ISOLATION AND CHARACTERIZATION OF SYMBIOTIC BACTERIA FROM  
*HETEROTERMES INDICOLA***

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*Heterotermes indicola* is a semi-relict specie, most significant group of subterranean termites and cosmo-tropical in the division, records from all the zoogeographical regions, besides the Palaearctic region. It is found in humid and drought areas. Termite depends on gut microorganisms for the digestion of wood and these microorganisms play a cardinal physiological role like decomposition of cellulose and hemicelluloses, acetogenesis, hydrogenesis, methanogenesis, reduction of sulfur and fixation of nitrogen. The symbiotic microorganisms in termite gut, include, protozoa,

bacteria, archaea and fungi. In the present study we isolated five bacterial species from the termite gut, *Heterotermes indicola*. The isolated bacteria were gram negative, facultative anaerobes and aerobic in nature. These include, *Shewanella putrefaciens*, *Pseudomonas luteola*, *aeruginosa* and other species and *Proteus mirabilis*. The biochemical tests also confirmed the presences of above mentioned five bacterial species. The phylogenetic techniques were applied on two of the bacterial cultures, *Pseudomonas aeruginosa* and *Pseudomonas spp.*, by using 16S rRNA clone. This was the prime information on the appearance of bacteria living with or without the presence of oxygen in the hindgut of the termite. The results of this study denoted that the function of such facultative bacteria and aerobic bacterial species in the gut of *Heterotermes indicola* is to eliminate oxygen and establish an anaerobic environment for the other anaerobic microorganisms, such as protozoa (the primary source of cellulose). The effect of bacteria on cellulose and lignocelluloses material decomposition in the gut of termite has ever been a subject of discussion.

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## 6. MOLECULAR BIOLOGY

### EVALUATION OF GENE TRANSCRIPT RATIO OF DNA-R OF BANANA BUNCHY TOP VIRUS INFECTED BANANA BY USING qPCR

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*Banana bunchy top virus* (BBTV) is the most important reasonably priced virus which badly effect banana production. Present Study was conducted to find out gene to transcripts ratio of BBTV in infected banana. It's important to know about the dynamics and relationship of the transcripts expressed from the major DNA components of BBTV to recognize the important genes helping the virus to cause infection in plant. In ordered to achieve these objectives DNA was extracted by using CTAB method. Extracted DNA were used for PCR amplification using primers designed against BBTV genome .RNA isolation was carried out by using SV total RNA isolation system (promega, USA) according to manufacturer's instruction. Extracted RNA was subjected to cDNA synthesis using superscript III one step quantitative RT-PCR system The results of work prove the evidence that BBTV components vary in their concentration in the cell and BBTV Rep interactions determine the extent of replication, suggesting that BBTV Rep not only help in replication of BBTV genome but also act as regulator of replication for fine tuning of gene to transcript ratio for controlling early and late gene expression. This information will help in the understanding of life cycle of BBTV with respects to gene expression and will provide the opportunities to dissect this process by some gene silencing tool to mitigating this disease effectively.

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### DETECTION OF GENOTOXIC EFFECTS OF WATERBORNE BIFENTHRIN IN PERIPHERAL BLOOD ERYTHROCYTES OF FRESH WATER FISH, *CATLA* *CATLA* USING COMET ASSAY

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Bifenthrin (Pyrethroids) belongs to the most commonly used pesticides throughout the world. Their extensive usage is a threat to the natural environments including aquatic ecosystems. Although bifenthrin are rapidly degraded in soil and plants, but they are extremely toxic to the fish. Keeping in view the high sensitivity of fish to bifenthrin, the present study was conducted to detect the genotoxic effects of waterborne

bifenthrin in peripheral blood erythrocytes of freshwater fish, *Catla catla* using Comet assay. The 96-hr LC<sub>50</sub> of bifenthrin for the fish was determined as 5.51±1.00 µg L<sup>-1</sup>. Fish were exposed to four sub-lethal concentrations viz. 10, 20, 33 and 50% LC<sub>50</sub> of waterborne bifenthrin along with positive (Cyclophosphamide) and negative control groups, separately, for 30 days. The DNA damage in terms of types of DNA damage (Type I – IV) from which percentage of DNA damaged cells, genetic damage index (GDI) and cumulative tail length of comets (µm) in peripheral erythrocytes of *Catla catla* exposed to different sub-lethal concentrations, with negative and positive control groups were also determined. The erythrocytes of *Catla catla* showed significantly variable effects of different concentrations of bifenthrin on percentage of DNA damaged cells, GDI and cumulative tail lengths of comets at p<0.05. The 50% LC<sub>50</sub> exposure of waterborne bifenthrin showed significantly higher DNA damage in terms of percentage of DNA damaged cells and GDI followed by that of positive control, 33%, 20%, 10% of LC<sub>50</sub> and negative control. However, cumulative tail lengths of comets were significantly maximum due to positive control followed by that of 50% and 33% LC<sub>50</sub> exposure of bifenthrin. Significantly positive concentration dependent increase in DNA damage was also observed during present investigation. This study also reveals that bifenthrin is very toxic to the fish and Comet assay can be used as useful tool for the determination of genotoxic effects of pesticides on fish.

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#### PROTEIN-PROTEIN INTERACTION BETWEEN THE SHORT AND LONG FORM OF GRG FAMILY OF CO-REPRESSORS

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The Grg family of corepressor proteins lacks DNA-binding domain and these are recruited to the promoter region by interacting with DNA-binding transcription factors. Recruitment to the promoter by DNA-binding transcriptional factors results in transcriptional repression. There are five member of Grg proteins namely Grg1-5. Grg1-4 are the long forms and Grg5 is the short form of Grg family of corepressor. AES is the Human homologue of mouse Grg5, and TLE1-4 are the human homologue of mouse Grg1-4. Both the short form Grg5 and long forms Grg1-4 repress the transcription activity of transcriptional factors. However, the short Grg5 has also been shown to act as derepressor of transcription. No conclusive mechanism is given as to how Grg5 can act as derepressor. The possible mechanism could be the protein-protein interaction of the long and short form of Grg proteins. However, no evidence is available from protein-protein interaction assays. COS7 cells were transiently transfected using electroporation with myc-Grg5 and GFP-Grg1 and GFP-Grg4. 20 hours after transfection, cells were processed for immuno-assay. Anti-myc antibody was used to detect myc-Grg5. Nucleus

was stained with DAPI (Vector). Fluorescence signals were observed under epifluorescence microscope and recorded by digital camera (Nikon). The merging of the images was carried out using Adobe Photoshop Elements 5.0. Grg5 is the short of Grg family of corepressors. Grg5 lack Nuclear localization signals. myc-Grg5 when transfected alone was localized both in cytoplasm and nucleus. However, cotransfection with either GFP-Grg1 or GFP-Grg4 caused complete translocation of Grg5 into the nucleus. GFP vector was used as control and cotransfection of Grg5 with GFP vector did not change the localization of Grg5. This suggests the interaction of Grg5 with Grg1 and Grg4. Another observation was colocalization of both the short form Grg5 and long forms Grg1 or Grg4 into the nuclear bodies. This suggests that Grg5 and Grg1-4 proteins are sequestered into the nuclear bodies, which might be the possible mechanism of repression.

**pH NEUTRALIZING EFFECT OF *KLEBSIELLA PNEUMONIAE* STRAIN MBZ6 IN ACIDIC MEDIUM AND MOLECULAR CHARACTERIZATION OF ITS TRANSCRIPTIONAL ACTIVATOR (*cadC*) GENE**

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*Enterobacteriaceae* have developed amino acid dependant efflux systems for Acidification Tolerance Response (ATR). Cad System is one of the four Acid Resistance (AR) systems possessed by different members of *enterobacteriaceae*. Cad system gives lysine dependant acid tolerance, it comprises of an operon; *cadBA* and its positive regulator; *cadC*. Transcriptional activator gene (*cadC*) was amplified by PCR using manually designed primers. The amplified product was purified and sent to Macrogen Korea for sequencing. A sequence of 1800 bases was received that contained full length *cad* gene (1569bp). BLAST results showed 99% homology with already reported *cadC* of *Klebsiellapneumoniae*. The translated sequence indicated CadC with 522 amino acids. Hydropathy graph shows this protein has both hydrophobic and hydrophilic domains inferring that it has both membrane embedded and cytosolic domains. Secondary structure of CadC revealed that it is made up of a single long polypeptide with multiplex configuration; helices, sheets, turns and coils. The complexity increases moving from N-terminal towards C-terminal. Growth pattern of *Klebsiellapneumoniae* strain MBZ6 show that it has ability to grow up to pH 4 under stressed conditions. pH neutralizing ability was studied and results indicated that *Klebsiellapneumoniae* strain MBZ6 neutralized pH 4 and 5 in 10 and 8 hrs respectively but no change in media having pH 3 was observed.

### ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISM IN *CDKLI*, *PPARG* AND *IL6* GENE WITH TYPE 2 DIABETES IN PUNJABI POPULATION OF PAKISTAN

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Pakistan is a developing country and diabetes prevalence has reached up to 11%. The current study was designed to reveal the association of genetic polymorphism with type 2 diabetes (T2D) and the interaction of genes with demographic characteristics. Nested PCR was used for DNA amplification. Genotyping was performed by One Label Extension method and also confirmed by sequencing. The selected genes play a role in dysfunctioning of  $\beta$ -cells decreasing the insulin production involved in the predisposition of T2D. The genetic variants were in *CDKLI* (cyclin-dependent kinase 5 regulatory-subunit associated protein 1- Like 1, rs7756992 A > G), *PPARG* (peroxisome proliferator-activated receptor-g2, rs1801282 G > C) and *IL6* (Interlukin-6, rs1800796 C > G). Single site analysis indicated that rs1801282 in *PPARG* and rs1800796 from *IL6* were significantly associated with T2D [ $P = 0.004$ , OR = 2.188(1.254, 3.815);  $P = 0.0001$ , OR = 0.394 (0.265, 0.584)]. Genotype analysis showed the association of rs5443, rs1801282 and rs1800796 with the onset of T2D ( $P < 0.05$ ) where the risk genotypes were TT, CG and GG respectively. Linear regression analysis between demographic characteristics and genotypes showed a positive association of CC from rs1800796 with family history of diabetes ( $P < 0.05$ ). The CT genotype from rs7756992 was associated to complications of T2D ( $P < 0.05$ ). Our study concluded that ProAla in *PPARG*, *IL6* and *CDKLI* genes depicted a role in predisposition of T2D. The gene *IL6* and *CDKLI* were gender specific in Punjabi population of Pakistan.

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### CLONING, EXPRESSION AND CHARACTERIZATION OF CHITINASE FROM *BACILLUS*

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Chitin is a linear polymer of  $\beta$ -(1, 4)-linked N-acetylglucosamine (GlcNAc)



sugar while the Chitinases are the enzymes that are responsible for the breakdown of chitin. Various *chitinase* genes from different microorganisms have been amplified and are expressed in different hosts. The ultimate uses of these genetically manipulated organisms have been discussed by various researchers. *Bacillus thriugenesis* produce Chitinase as a major carbon source when it is supplemented with a medium containing colloidal chitin. The present study is focused on the cloning and expression of hydrolytic enzyme *i.e* Chitinase in *E.coli* from *Bacillus thriugenesis*. In this context gene of size 1.5kb was selected and primers were designed. The gene sequence was amplified by PCR followed by ligation of PCR product into pTZ57R/T cloning vector. This recombinant vector was transformed to competent cells of *E.coli* (DH5 $\alpha$ ) that were prepared by CaCl<sub>2</sub> treatment. These plasmids were restricted with *Nde*I and *Hind*III. The reaction mixture was run on agarose gel and 1.5kb band was cut and purified. pET21 was also isolated and restricted with same enzymes followed by transformation. The expression is under process in which both the purified gene and the expression vector will be ligated using T4 DNA ligase. After an overnight incubation the ligation mixture will be used to transform in competent cells of *E.coli* (DH5 $\alpha$ ). Colonies that will be obtained after transformation will be screened for gene of interest. Plasmid will be isolated from positive clones and cloning will be confirmed with single restriction. Recombinant plasmid will be transformed to *E.coli* BL21 codon plus. The recombinant plasmid will be expressed using IPTG and expression will be analyzed by SDS-PAGE. Band of 71kD will confirm the successful transformation.

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#### CHARACTERIZATION OF EXTRACELLULAR CHITINASES FROM MESOPHILIC *BACILLUS THURINGIENSIS* STRAINS

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Chitin is an insoluble linear  $\beta$ 1-4 linked polymer of N-acetylglucosamine and a common constituent of the fungal cell wall and exoskeleton of arthropods. Chitin along with its derivatives is of huge economic interest because of its biological activity as well as its biomedical and industrial applications. Due to its abundant annual production it is considered as a renewable energy resource. In the present study, 13 *Bacillus thuringiensis* were taken and screened on colloidal chitin media prepared from shrimp shell for chitinolytic activity. All strains of *Bacillus thuringiensis* were found to have chitinolytic activity. The chitinolytic strains were screened for maximum activity at varying pH. Chitinase activity was assayed as the amount of N-acetylglucosamine released in U/ml using the DNS assay method. The maximum activity ( $0.229 \pm 0.001$  U/ml) was shown by CMBL-Bt4 after 4 days of incubation at 37°C (pH 7.0) when medium was supplemented with 1% colloidal chitin. PCR was employed to amplify the *chitinase* gene from SBS-Bt1, SBS-Bt3, SBS-Bt5 and SBS-Bt6 by using a specific pair of primers. The amplified gene was then cloned into pTZ57R TA cloning vector and transferred in *E.coli*. The

sequenced genes of SBS-Bt1 (Accession No: HG792450), SBS-Bt3 (Accession No: HG792451), SBS-Bt5 (Accession No: HG792449) and SBS-Bt6 (Accession No: HG792452) consist of 1195, 1103, 1212 and 1150 nucleotides respectively encoding 360 residues. The *chi* gene sequences of the studied strains shows 95% homology to that of *Bacillus cereus chiA* (AB041931.1), *Bacillus cereus* strain 49 *chiA* (GU134904.1) and 90% similarity with *Bacillus thuringiensis* serovar *colmeri chiA* (EF103273.1).

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**MOLECULAR CHARACTERIZATION AND COPPER INDUCED  
TRANSCRIPTIONAL ANALYSIS OF *copA*, A COPPER TRANSLOCATING p-  
TYPE ATPase, OF *KLEBSIELLA PNEUMONIAE***

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Copper is an essential transition metal playing vital role in many biological processes. However it becomes hazardous at higher concentrations. Life has developed strategies to maintain copper concentrations within biological range. Various genetic determinants are involved in this process. In the present study, role of CopA, a Cu(I) translocating p-type ATPase, has been explored in copper resistance in *Klebsiella pneumoniae*. *copA* (2.93 kb) was amplified, cloned and subjected to sequencing. Comparative analysis of the deduced CopA sequence revealed its phylogenetic relationship with homologous sequences retrieved from gene databank. Topological analysis showed that CopA was a membranous protein with six transmembrane helices. Modeling of three dimensional structure and Conserved Data Domain analysis revealed that CopA of *K. pneumoniae* is comprised of four domains; two HMA, one E1-E2-ATPase and one HAD-like domain. Regulation of *copA* in *K. pneumoniae* was monitored at transcriptional level over a range of copper (0-5 mM). Quantitative analysis through real time PCR revealed a significant increase in mRNA level of *copA* in the presence of non-lethal Cu<sup>++</sup> concentrations that further increased in the presence of sub lethal ones. A massive increase was observed when cells were grown in the presence of 4 mM copper. However fold increase of transcript level was comparatively less in the presence of 5 mM Cu<sup>++</sup>, proved to be lethal for cell survival. Comparative analysis of *copA* transcripts along with *cueO* and *cusCFBA* showed that *copA* plays key role in copper homeostasis and resistance in *K. pneumoniae*. Cloned *copA* was expressed in *E. coli* BL21 C<sup>+</sup> using pET21a<sup>+</sup> as expression vector. Maximum expression of CopA was found with 4 hours induction over a broad range of IPTG. CopA was found to be expressed in insoluble form however partially soluble protein was obtained when cells were grown at low temperature.

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### DNA SEQUENCING BASED IDENTIFICATION OF FRESH WATER TURTLES FOUND IN RAWALPINDI-ISLAMABAD AREA OF PAKISTAN

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DNA-based characterization and identification of fresh water turtle species for phylogenetic analysis as well as forensic identification is widely being carried out with the help of polymerase chain reaction (PCR) with DNA sequencing method. In the present study, three species of fresh water turtles *i.e.*, *Lissemys punctata*, *Nilssonina gangetica* and *Pangshura smithii* found in Rawalpindi – Islamabad area were identified and characterized by using PCR method. Under studied fresh water turtles *i.e.*, *Lissemys punctata*, *Nilssonina gangetica* and *Pangshura smithii*, were grouped into three clusters by constructing Dendrograms based on Cytochrome b gene, made by Maximum Parsimony and Neighbour-Joining methods. It is inferred that these species were 89-96% resembled with Southeast Asian freshwater turtles in general and to Indian turtles in particular. The cluster analysis revealed these three clades representing *L. punctata*, *N. gangetica* and *P. smithii* representing three genera, respectively. Clades I and II consisting of *L. punctata* and *N. gangetica* and have close resemblance as compared to Clade III comprising of *P. smithii*. Tajima's neutrality test was performed using a MEGA version 5.1 which revealed 246 segregating sites out of 656 bases in all three sequences. The nucleotide diversity index ( $\pi$ ) was estimated to be 0.262195 by Tajima's neutrality test. The results indicated the use of molecular techniques to be an efficient and more reliable to identify fresh water turtle species in general and endangered species in particular and to keep in their proper taxonomic position.

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### MOLECULAR CHARACTERIZATION OF ARSENIC RESISTANCE PROTEINS IN *KLEBSIELLA PNEUMONIAE* CC

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Arsenic is a potent toxic metalloid. Its pentavalent ( $As^{5+}$ ) and trivalent ( $As^{3+}$ ) ions resemble phosphate ions therefore it cause inhibition of many cytoplasmic reactions. Current study is based on the study of genetic basis of *ars* gene in chromosome based *ars* operon in *Klebsiella pneumoniae* CC. This operon consists of three genes, *i.e.*, *arsR*, *arsB* and *arsC*. *arsR* (330bp), *arsB* (1290bp) and *arsC* (426bp). All genes were amplified by means of PCR from the genomic DNA of *Klebsiella pneumoniae* CC, cloned in pTZ57R and get sequenced. Expression primers were designed, nested *ars* genes were amplified,

each having *Nde* I at 5' site, a noncutter enzyme of gene at 3' and ligated in pET21a. Recombinant vector was subjected to an expression system of *E.coli* (BL21C<sup>+</sup>). *ArsR* and *arsC* are soluble proteins whereas *arsB* is an insoluble protein. Expression of all proteins was optimized for IPTG concentration, temperature and pH. *arsB* gene was ligated in pET28a for the purpose of purification by means of single step his-Nickel affinity column chromatography, whereas *ArsR* and *ArsC* proteins were subjected to affinity chromatography for the purpose of purification. *ArsR* is a regulatory protein in nature having 109AA. It controls the expression of whole *ars* operon. It has a molecular mass of 12.3Kda and a pI value of 9.38. *ArsB* is a structural protein of membrane consisting of 430AA and acts as arsenical pump. Its size is 42.15Kda and a pI value of 6.91. *ArsC* is a structural protein that acts as an enzyme arsenate reductase. It is 142AA in length having a molecular mass of 16.7Kda with pI value of 4.95. It is actively involved in conversion of arsenate to arsenite.

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**CHARACTERIZATION OF MULTI-COPPER OXIDASE (CueO) OF  
*KLEBSIELLA PNEUMONIAE* AND ITS TRANSCRIPTIONAL REGULATION IN  
RESPONSE TO COPPER**

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Multi-copper oxidase (CueO) is an enzyme in periplasmic space of *E. coli* believed to play role in detoxification of copper (oxidizing Cu I to less harmful Cu II) contributing to survival in excess copper environment. However, it is needed to decipher its mechanism of action by further studies. In this study, we have explored CueO of *Klebsiella pneumoniae* KW which is resident of highly contaminated water of local area. Multi-copper oxidase gene (1.6kb) of the above mentioned strain was cloned in DH5 $\alpha$  through pTZ57R cloning vector and subjected to sequencing. Having confirmation of CueO on the basis of sequencing, CueO was expressed in BL21 cells using pET28a as expression vector. Conditions of expression were optimized for various parameters including inducer concentration (IPTG) and induction time. Optimum level of expression was found at 0.05mM IPTG concentration. On time course, it was found that expression of protein increased with increase in duration of induction upto 8h beyond which it remained constant. CueO enzyme was expressed as inclusion body. The protein was made to express in soluble form by growing bacterial cells at 20°C for 36 hours. CueO protein of 62kDa was purified to homogeneity by nickel affinity chromatography for further characterization assays. Expression of CueO was measured through real time PCR in response to varying concentrations of copper. It was found that CueO level of expression increased upto 3mM of Cu<sup>++</sup> indicating active role of the gene in copper resistance. However, with further increase of copper stress, a lesser increase in CueO transcript level was observed in a hyperbolic manner. There is further need to test the enzymatic activity towards various phenolic substrates and find its biotechnological applications.

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**CHARACTERIZATION AND QUANTITATIVE ANALYSIS OF *cueR*  
THROUGH REAL TIME PCR IN *KLEBSIELLA PNEUMONIAE***

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*Klebsiella pneumoniae* is an important member of enterobacteriaceae. In this family, various genetic elements in form of Cue (copper efflux) and Cus (copper sensing) regulons are responsible for copper homeostasis and resistance. Cue regulon comprised of CopA and CueO is regulated by CueR, a MerR-like transcription activator protein. *cueR* (478 bp) from *K. pneumoniae* KW was cloned in pTZ57R cloning vector and subjected to sequencing. Three dimensional structure of CueR homodimeric functional form was constructed that had a bisymmetrical form, a characteristic of transcription activators. Later *cueR* was cloned in pET21a expression vector and CueR was expressed in BLC21 C<sup>+</sup> induced with IPTG. Fractionation of the induced sample revealed that CueR expressed as soluble form. Optimal conditions for CueR expression were found to be 37°C with 0.02 mM IPTG induction for 4 hours. To exposed its role for regulation of Cue regulon in response to various Cu<sup>++</sup> concentrations, transcript level of *cueR* was quantitatively studied over a range of copper. Comparative analysis through real time PCR revealed that *cueR* possessed a basal level that was well sufficient also in the presence of non-lethal amounts of copper in the medium as no change was observed when cells were growing under such conditions. However an increase was found in the presence of sub-lethal concentrations showing that more amount of the transcriptional activator was required for enhanced expression of components of Cue regulon.

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## 7. PHYSIOLOGY

### BLOOD AND SOFT TISSUE LEAD LEVELS OF GOATS GRAZING AROUND THE UNCONTROLLED BATTERY RECYCLING SMELTERS

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In developing countries including Pakistan tonnes of lead acid batteries are recycled annually in uncontrolled manner for extraction of lead. Most of such smelters operate in slums or outskirts of major cities paving way for equal exposure of toxic metal to humans and animals. In the present study clinical signs of lead poisoning were noted in the goats grazing in the meadows surrounding battery recycling smelters in the outskirts of city of Hyderabad, Sindh, Pakistan. The laboratory studies were carried on blood and tissue samples derived from kidneys and cerebrospinal parts of goats to detect the lead levels. Results showed the lead level ranging between 65-105 µg/dl (blood) 48-59 µg/kg (kidney) and 40-53 µg/kg in cerebrospinal tissues. The chemical analysis of soil samples and grasses for lead was also conducted and the findings revealed presence of lead in the range 234- 3330 mg/kg with direct correlation ship with body lead burden of goats. The aerial parts of grass were found to contain lead in the range 1516- 2230 mg/kg whose 70% could be removed if washed with water. The overall results showed that highest mean lead levels were detected in the, blood followed by kidney feces and cerebrospinal tissues. The lead content in soil samples collected at different distances from smelter showed inverse relationship distance where as the grass samples on the other hand showed insignificant variations with distance from smelter. The study proved to identify well defined chain of lead transfer involving recycling smelter, soil, grass, grazing cattle with ultimate end in human beings.

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### BLOCKING THE BRAIN IL-1 RECEPTORS DECREASES CENTRAL NITRIC OXIDE PRODUCTION AND INCREASES VASOPRESSIN SECRETION IN THE LATE PHASE OF SEPSIS

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During sepsis, the entry of pathogens into systemic circulation causes numerous physiological alterations, including increased plasma nitric oxide (NO) levels. Higher

plasma NO levels contribute to vasodilatation, hypotension, may decrease vasopressin secretion in the late phase of sepsis and ultimately lead to death. At present, it is not known whether a progressive increase in NO production, as seen in plasma, also occurs in the brain, and whether such NO production could be affected by the interleukin (IL)-1/IL-receptor (IL1r) pathway. In this study, we investigated the correlation of plasma and cerebrospinal fluid (CSF) NO levels, and we also studied the effect of IL1r antagonist (IL1ra) treatment on sepsis-induced alterations in plasma and central NO levels, as well as vasopressin secretion. In male Wistar rats previously i.c.v injected with an IL-1ra (9- and 18-nmol/animal) or vehicle (PBS), sepsis was induced by cecal-ligation and puncture (CLP). CSF and blood samples were collected from each group (n=6-8/group) at 1-, 2-, 4-, 6- and 24-h after surgery. NO levels were measured by a chemoluminescence assay. NO levels were significantly ( $P<0.05$ ) increased at post-CLP 6- and 24-h. IL-1ra administration did not significantly alter NO concentrations in CSF in first 6-h as compared to vehicle treatment and untreated animals. But after 24 h, NO levels were significantly ( $P<0.02$ ) diminished in IL-1ra-treated animals as compared to the vehicle group. The IL-1ra treatment did not affect plasma NO levels. Moreover, vasopressin levels and the survival rate were significantly higher in IL-1ra treated rats. In conclusion, our results showed that blocking the IL-1-IL-1r signaling pathway by central IL-1ra administration decreased CSF NO levels and increased vasopressin secretion in the late phase of sepsis, which may be beneficial for the survival septic rats.

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#### **EFFECTS OF EXOGENOUS OXYTOCIN ON MILK PRODUCTION, COMPOSITION, REPRODUCTIVE HEALTH AND ITS RESIDUAL EFFECTS IN RUMINANTS**

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Oxytocin is a hormone released from the posterior pituitary gland. It causes the contraction of the myoepithelial cells around alveoli and small ducts of the mammary gland. The discovery of oxytocin and its role in the neuro-hormonal milk ejection process, allowed for managing the milking process with an exogenous hormone. Oxytocin increases milk yield, it increases milk production may not be caused by removal of residual milk but by increased gland output of milk. Its receptor blockage causes inhibition of milk ejection. It also improves the persistency of lactation. Intramuscular OT injection causes a long increase of OT blood levels and prolonged myoepithelial and alveolar contraction thus it increases the milk yield. Along with this exogenous oxytocin have effects on milk composition especially for fat, protein, lactose and mineral concentrations. It influences the mammary metabolism and cell maintenance in addition to its well-established physiologic role in milk ejection reflex. Effect of oxytocin is not manifested through an effect on cell remodeling. Different reproductive anomalies like follicular ovarian cyst, corpus-luteum cyst, anestrous, delayed age at

puberty, abortions, difficult births (dystocia), dead fetuses, retention of placenta and repeated estrus cycles was observed effects of oxytocin administration. Oxytocin whether secreted endogenously or administered exogenously, produces the desired effects within minutes and is metabolized rapidly into inactive products. If at all oxytocin is secreted in the milk and is ingested along with milk, it is degraded by the gut enzymes and cannot reach blood circulation in biologically active form so there seems to be no harm in consuming milk from oxytocin-treated dairy animals.

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### REPRODUCTIVE MANAGEMENT IN CROSSBRED CATTLE THROUGH ADMINISTRATION OF GnRH, hCG AND PROGESTERONE AFTER ARTIFICIAL INSEMINATION

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The present study was designed to know the effect of administration of GnRH, hCG and progesterone after A.I. on conception rate and serum progesterone concentrations in cross bred cows. A total of 40 lactating cross bred (Friesion x Sahiwal) cattle of different lactation were selected from the herd of Military Farm located in Multan Cantonment. These animals were divided in to 4 groups (A, B, C, D) comprising of 10 animals in each group (n=10). Group A was given a single intramuscular injection of GnRH at the dose rate of 100 µg on day 7 after artificial insemination whereas Group B was given a single intramuscular injection of hCG at the dose rate of 3300 IU on day 7 after artificial insemination. Cows of Group C were given an intramuscular injection of progesterone at the dose rate of 50 mg/kg body weight for 7 days starting on day 7 after artificial insemination and Group D was given no treatment and served as control. Blood sampling from all experimental cows was done at the start of treatments and 7 days later for progesterone estimation. All cows were examined for pregnancy by palpation per rectum of the uterus and its contents for detection of an embryonic vesicle at 40 ± 1 d after AI, and pregnant cows were re-examined 4 week later at 68 ± 1 d after AI. The mean conception rate was significantly higher ( $P < 0.05$ ) in cows of group B and C as compared to control where as non-significant differences ( $P > 0.05$ ) were observed between group A and D. Non-significant difference ( $P > 0.05$ ) in conception rate was also observed between group B and C. There was non-significant difference ( $P > 0.05$ ) in serum progesterone concentrations between group A, B and C during 7 and 14 days after artificial insemination (AI). Whereas there were significant differences in serum



progesterone concentrations ( $P < 0.05$ ) between treatment (A, B & C) and control group cows. Progesterone concentrations were significantly higher in treated groups of cows when compared with control group. It was concluded, that hCG or progesterone could be used as a method for improving fertility rate in crossbred cattle.

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**STUDIES ON PRE-OVULATORY STAGES IN THE OVARY OF THE FISH,  
*OREOCHROMIS MOSSAMBICUS***

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An attempt was made to study the pre-ovulatory stages in the ovary of *Oreochromis mossambicus*. The fish mean body weights  $44.2 \pm 8.0$  were collected from the adjoining waters of Jamshoro Sindh during the early August to late September. The ovaries of the each fish were dissected out and processed for histological examination. The study of the ovarian sections during the pre-ovulatory phase reveals that the ovary of the fish in early August contained gravid oocytes in majority. However, few developing oocytes were also visible. After 20 days the ovaries of fish collected were in advanced condition. The oocyte membrane of majority follicle disintegrated and they were ready for ovulation. Beside this the ovarian wall became thin and also ruptured from one side indicating the start of spawning phase. It has also been noted that the oocytes were present in different stages of development and are released as the final maturation takes place. The ovaries processed after first spawning (early October to early December) reveals that the oocytes which were left after the onset of spawning achieved final maturation and released after ovulation. However, the ovarian wall which became thin at the time of spawning starts recovering. Second batch of the oogenic material was noted by the appearance of the stem cells located in the ovarian folds. These cells were found developing as a second batch. The second batch of oocytes when reaches the final maturation it is followed by the third batch and so on. It can be said that *Oreochromis mossambicus* breeds in clutches hence called as the multi spawner fish.

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**CARDIOVASCULAR RISK FACTORS IN PRE AND POST MENOPAUSAL  
WOMEN IN URBAN AREAS OF HYDERABAD**

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Cardio vascular risk factors are the leading cause of morbidity and mortality in post menopausal women. Million people die each year due to Cardiovascular diseases. Number of studies reports the high ratio of post menopausal women suffering from Cardiovascular diseases. No study is conducted in Pakistan to find out the prevalence of Cardio vascular risk factors in pre and post menopausal women. Crosses sectional study

was carried out on 270 urban women out of them 150 were in pre-menopause and 120 were in post menopause. The data was collected on structured questioner followed by the measurements of Wais Circumference, Hip Circumference, Waist-Hip Ratio, BMI, Blood Pressure, Venous blood collected for Fasting blood glucose and for lipid profile while subjects were on fasting an overnight. Histories of Hormones Replacement Therapy and for smoking were also taken. The mean age of premenopausal women was  $42.30 \pm 5.85$  and mean age of post menopausal was  $55.25 \pm 2.76$ . Assessment of CVD risk factors revealed that fasting Blood Glucose, Systolic& Diastolic blood pressure, Triglycerides, Total- cholesterol, Very Low Density Lipoprotein, Waist-Hip Ratio were significantly higher (p-values  $<0.05$ ) in post menopausal women. However, no significant difference (p-values  $>0.05$ ) was found in waist circumference, Body Mass Index, Low Density Lipoprotein, High Density Lipoprotein of pre and postmenopausal women. Assessments of CVD risk factors show that post menopausal women are at more risk for developing Cardio-vascular diseases. This study suggests that postmenopausal women are higher risk of developing CVDs than premenopausal women.

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## 8. TOXICOLOGY

### ACCUMULATION AND REDUCTION OF LEAD AND CHROMIUM BY BACTERIA

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Heavy-metal pollution represents an important environmental problem due to the toxic effects of metals, and their accumulation throughout the food chain leads to serious ecological and health problems. Both Chromium and Lead have adverse environmental effects. Two bacterial species *Bacillus subtilis* and *Pseudomonas aeruginosa* were used. Results showed that both the species absorbed and reduced the chromium and lead at all the initial concentrations. *B. subtilis* showed maximum absorbance (5.14 mg/g) of chromium and (392 mg/g) of lead, in case of *P. aeruginosa* the maximum absorbance (5.08 mg/g) of Cr and (3.06 mg/g) of Pb were shown after 10 hrs of contact time. The maximum reduction of Cr and Pb by *B. subtilis* is 53.49% and 64.70% respectively, while the maximum reduction by *P. aeruginosa* is 93.38% and 92.27% respectively. The results showed that *P. aeruginosa* has greater potential of reducing the chromium and lead as compared to *B. subtilis*.

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### TESTICULAR MICROMETRIC AND HISTOPATHOLOGICAL ALTERATIONS OF CHROMIUM AND THEIR AMELIORATION UPON MORUS NIGRA FRUIT PULP EXTRACT IN MICE

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Testicular histopathology and histometry of seminiferous tubules and various spermatogenic cells against short-term hexavalent chromium, Cr (VI), exposure and their ameliorations upon post treatment of *Morus nigra* fruit pulp extract were studied in mice. There were three groups of animals each named as control (C), chromium (Cr), and chromium-Morus (Cr-M). Hexavalent chromium (50ppm from potassium dichromate) was provided in drinking water to the Cr and Cr-M group animals for ten days, additionally the animals in Cr-M group received 0.2ml Morus fruit pulp extract- twice a day for the next 5 days while the control group was maintained on Cr free water throughout the study period. Animals were sacrificed to remove testes on day 16. One testis from each animal was routinely processed for histological preparations while the other was used for the preparation of testicular smears. The pathological signs of Cr

exposure include necrosis of interstitial tissues. Rupture of basement membrane of various seminiferous tubules, extremely scanty and scattered spermatogonia, primary spermatocytes and dislodged spermatogenic cells (including spermatozoa and spermatids), increased number of club headed sperms and lack of tail in many of the dislodged spermatozoa especially of parrot beak headed type. These pathological alterations were recovered effectively on Morus pulp extract treatment. Histometric findings show significant ( $p < 0.05$ ) decline in mean cross-sectional area of the seminiferous tubules, number of embedded spermatozoa and relative abundance of parrot beak headed spermatozoa. On the other hand, the number of club headed and dislodged spermatozoa were significantly higher in Cr to that of the control group. Similarly, significant alterations in spermiometry were noted in Cr treated group compared to control. These histometric and micrometric changes were found ameliorated in Cr-M group. Results show that Morus pulp extract has got curative properties against testicular histopathological and micrometric alterations of Cr exposure.

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#### **DEVELOPMENTAL DEFECTS PRODUCED BY PRENATAL EXPOSURE OF DICLORAN IN MICE**

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Diclofenac sodium was tested to find out for its teratogenic and embryotoxic effects on the developing mice. For this purpose different concentrations 0.83, 1.66, and 3.33mg/g B.W. were given orally to pregnant mice once in a day during days 6-12 of gestation. The treated gravid mothers were anaesthetized on day 18 of gestation and fetuses were removed for morphological and morphometric analysis. The morphological studies showed the developmental anomalies such as hyperextension of forelimbs, low set arm, drooped wrist, meromelia, adactyly, anophthalmia, hydrocephaly, intra uterine growth retardation, kinky tail, resorbed fetuses, subcutaneous hemorrhages and subcutaneous edema. The incidence of abnormalities were intensified in higher dose groups. The morphometric analysis showed a significant ( $p < 0.05$ ) decrease in mean body weight of fetuses, crown-rump length, head circumference, eye circumference, forelimb length, hind limb length, snout size and tail length of recovered fetuses as compared to controls. Histologically, observed defects were found in cardiogenesis and hepatogenesis. The results clearly indicate that Diclofenac sodium is potent teratogen for developing mice fetuses, which may be equally harmful for human development. It is clearly shown that Diclofenac sodium should not be taken by pregnant females especially during organogenetic period.

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**A STUDY ON THE ACUTE TOXICITY OF METALS MIXTURES FOR  
*CIRRHINA MRIGALA* AND *LABEO ROHITA***

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Acute toxicity tests play a significant role in environmental risk assessment and hazard classification because they permit us to estimate the relative toxicity of various chemicals in different species. So, this research work was planned to find-out the acute toxicity of 90-day old *Cirrhina mrigala* and *Labeo rohita* in terms of 96-hr LC<sub>50</sub> and lethal concentrations to waterborne metal mixtures of iron, zinc and lead. Experiments were performed at constant temperature (30 °C), pH (7.25) and total hardness (225 mg L<sup>-1</sup>) of water. Fish mortality was analyzed through Probit Analyses method with 95% confidence interval limits. Factorial Design and Tukey's/Student Newman-Keul test were also used to compare all treatments and species. The impacts of physico-chemical parameters on acute toxicity of mixtures were also observed. Both fish species showed differential toxicity for all selected metals mixtures at  $p < 0.05$ . *Labeo rohita* showed significantly higher tolerance against Zn+Fe (determined as LC<sub>50</sub>) and Pb+Zn (determined as lethal concentrations). *Cirrhina mrigala* were significantly more sensitive to three metals mixture Zn+Fe+Pb, followed by Pb+Fe, Pb+Zn and Zn+Fe mixtures. Regarding overall sensitivity of both species, Zn+Fe+Pb mixture appeared significantly more toxic to *Cirrhina mrigala* and *Labeo rohita* as evident from their low mean LC<sub>50</sub> (56.04, 82.91 mg L<sup>-1</sup>, respectively) and lethal concentration values (106.77, 139.28 mg L<sup>-1</sup>, respectively). In conclusion, the comparison of four mixtures revealed that, the mixture which contains three metals (Zn, Fe and Pb) showed synergistic effect on fish. These acute toxicity tests are expected to help us for the determination of water quality criteria and to assess the possible risk faced by our indigenous carps in natural water.

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**DETERMINATION OF TOLERANCE LIMITS OF *CTENOPHARYNGODON*  
*IDELLA* AND *HYPOPHTHALMICHTHYS MOLITRIX* TOWARDS  
WATERBORNE METALS MIXTURES**

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In natural waters, metals in mixture form are posing deleterious and toxic ill-effects in aquatic animals including fish. Therefore, the present project was designed to determine the tolerance limits of *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* towards mixtures of lead, zinc and nickel, in terms of 96-hr LC<sub>50</sub> and lethal

concentrations ( $\text{mgL}^{-1}$ ). Ten fingerlings (90-day old) of both fish species were separately exposed to metals mixtures viz. lead+zinc, lead+nickel, zinc+nickel and lead+zinc+nickel. The concentrations of metals mixtures were starting from zero and increased gradually ( $5\text{mgL}^{-1}$  separately for 96-hr) till 100% fish mortality. This mortality data was then used to estimate the  $\text{LC}_{50}$  and lethal concentration ( $\text{mgL}^{-1}$ ) values of each treatment by using Probit analysis method. The whole experiment was conducted under constant laboratory conditions of temperature ( $30^{\circ}\text{C}$ ), pH (7.25) and total hardness ( $225\text{mgL}^{-1}$ ). Water physico-chemical variables viz. temperature, pH, hardness, dissolved oxygen, total ammonia, carbon dioxide, electrical conductivity, sodium, potassium, calcium and magnesium were monitored periodically at 12-hour interval. Probit analysis revealed lower mean  $\text{LC}_{50}$  and lethal concentration ( $\text{mgL}^{-1}$ ) values for lead+zinc+nickel mixture of both fish species, indicating significantly higher toxicity of this mixture than that of lead+zinc, lead+nickel and lead+nickel. In conclusion, significant differences ( $p<0.05$ ) in the tolerance limits of both fish species were observed with *Hypophthalmichthys molitrix* being significantly more sensitive to all metals mixtures than that of *Ctenopharyngodon idella* (in terms of both  $\text{LC}_{50}$  and lethal concentrations). Correlation and step-wise regression analysis revealed varied relationships among physico-chemical parameters of test media and metallic ion concentrations. Metal mixtures concentrations in all test media showed significantly negative correlations with dissolved oxygen while the relationship with total ammonia, carbon dioxide, and electrical conductivity.

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#### **TOLERANCE LIMITS OF *CATLA CATLA* AND *LABEO ROHITA* FOR LEAD+COBALT MIXTURE**

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The present research work was conducted to investigate the tolerance limits of *Catla catla* and *Labeo rohita* against Pb+Co mixture. Water temperature ( $30^{\circ}\text{C}$ ), total hardness ( $225\text{mgL}^{-1}$ ) and pH (7.50) of the test media were kept constant throughout the acute toxicity trials. Each fish species was exposed, separately, to different concentrations of Pb+Co mixture, for a period of 96-hr. A group of ten ( $n=10$ ) fish were used, with three replicates for each test concentration of Pb+Co, mixture during 96-hr exposure period. The 96-hr  $\text{LC}_{50}$  and lethal concentration values of metal mixture for each fish species were computed through Probit analysis method. Factorial experimental design (RCBD) and Tuckey's Student Newman-Keul tests were employed to find-out statistical differences among various parameters under study. In order to find-out possible relationships among various parameters, regression and correlation analyses were also performed. The mean 96-hr  $\text{LC}_{50}$  and lethal concentrations of  $23.76\pm0.17$  and  $43.04\pm0.99\text{mgL}^{-1}$ , respectively, were estimated for *Catla catla*. The 96-hr  $\text{LC}_{50}$  and lethal concentrations computed for *Labeo rohita* were  $29.55\pm0.99$  and  $61.52\pm2.11\text{mgL}^{-1}$ , respectively. Regarding overall sensitivity of fish species towards Pb+Co mixture, *Labeo*

*rohita* were significantly more tolerant to metal mixture than *Catla catla*. Dissolved oxygen contents of the test media, used for both *Catla catla* and *Labeo rohita*, showed significantly inverse correlation with metal mixture concentrations while carbon dioxide and electrical conductivity showed significantly direct relationships with metallic ion concentration of test media used for both the fish species.

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**SUB-LETHAL EXPOSURE STRESS OF ALUMINIUM TO THE FISH, *CATLA CATLA*, *LABEO ROHITA* AND *CIRRHINA MRIGALA***

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The growth performance, condition factor, feed intake and feed conversion efficiency of 240-day three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* under chronic (1/3 of LC<sub>50</sub>) exposures of Aluminium (Al) were determined. The fish were fed with feed (34% DP and 3.00 Kcalg<sup>-1</sup> DE) to satiation daily. At termination of each trial, the fish were dissected and their organs viz. bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats isolated for the determination of Al concentrations. During growth trials under Al sublethal stress, *Catla catla* gained significantly higher weight, followed by that of *Cirrhina mrigala* and *Labeo rohita*. All the three control (un-stressed) fish species exhibited significantly better growth due to significantly higher feed intakes than those grown under sub-lethal concentrations of Al. The sublethal exposure of Al to the fish caused significantly variable accumulation of metal in the body organs of three fish species. Fish liver, kidney and gills accumulated significantly higher Al as 47.54±3.14, 44.99±3.11 and 35.23±1.17 µgg<sup>-1</sup>, respectively than the other organs of fish.

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**STUDIES ON ACUTE TOXICITY OF COBALT TO *TILAPIA NILOTICA*, *CYPRINUS CARPIO* AND *CTENOPHARYNGODON IDELLA***

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Acute toxicity tests were performed to determine the 96-hr LC<sub>50</sub> and lethal concentrations of cobalt (Co) for 60-, 90- and 120-day age groups *Tilapia nilotica*, *Cyprinus carpio* and *Ctenopharyngodon idella*. Toxicity tests were conducted under controlled laboratory conditions at constant pH (7.5), hardness (300mgL<sup>-1</sup>) and water

temperature (30C ). At the end of the each toxicity trail, the dead fish were dissected and their body organs viz. liver, kidney, gills, muscles, bones, fins, skin and muscles were isolated to determine the accumulation pattern of Co in these organs. Among three age groups, 60-day age group all the three fish species were significantly more sensitive to Co with the mean 96 LC<sub>50</sub> value of 48.28±14.99mgL<sup>-1</sup>, followed by that of 90- (60.49±17.28 mgL<sup>-1</sup>) and 120-day (77.66±22.78 mgL<sup>-1</sup>) age groups fish. The sensitivity of three fish species against Co in terms of 96-hr LC<sub>50</sub> and lethal concentrations followed the order: *C. idella* > *C. carpio* > *T. nilotica*. Significant variations in the sensitivity of all the three fish species viz. *T. nilotica*, *C. carpio* and *C. idella* appeared to be species specific. During these toxicity tests, the abilities of three fish species to bioaccumulate cobalt in their body organs varied significantly. However, all the age groups three fish species showed significantly higher accumulation in their kidney while it was significantly lower in muscles.

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#### DEVELOPMENTAL DEFECTS PRODUCED BY PRENATAL EXPOSURE OF DICLORAN IN MICE

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Diclofenac sodium was tested to find out for its teratogenic and embryotoxic effects on the developing mice. For this purpose different concentrations 0.83, 1.66, and 3.33mg/g B.W. were given orally to pregnant mice once in a day during days 6-12 of gestation. The treated gravid mothers were anaesthetized on day 18<sup>th</sup> of gestation and fetuses were removed for morphological and morphometric analysis. The morphological studies showed the developmental anomalies such as hyperextension of forelimbs, low set arm, drooped wrist, meromelia, adactyly, anophthalmia, hydrocephaly, intra uterine growth retardation, kinky tail, resorbed fetuses, subcutaneous hemorrhages and subcutaneous edema. The incidence of abnormalities were intensified in higher dose groups. The morphometric analysis showed a significant ( $p < 0.05$ ) decrease in mean body weight of fetuses, crown-rump length, head circumference, eye circumference, forelimb length, hindlimb length, snout size and tail length of recovered fetuses as compared to controls. Histologically, observed defects were found in cardiogenesis and hepatogenesis. The results clearly indicate that Diclofenac sodium is potent teratogen for developing mice fetuses, which may be equally harmful for human development. It is clearly shown that Diclofenac sodium should not be taken by pregnant females espacially during organogenetic period.

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### EFFECTS OF PROPOFOL ON HAEMODYNAMICS AND BLOOD PROFILE OF HUMAN

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The present study was conducted to assess the effects of Propofol on haemodynamic conditions and intracellular enzymes of pre and post anaesthesia blood samples in human. The blood samples of patients were collected before and after the treatment of anesthesia, from the District Hospital Gujarat, Pakistan. The systolic blood pressure, diastolic blood pressure, platelets, red blood cells, haemoglobin, alanine aminotransferase and creatinine were significantly different ( $P < 0.05$ ), whereas there was non-significant ( $P < 0.05$ ) effect on heart beat rate. It is concluded that Propofol has significant impact on hemodynamic parameters and blood profile in human, however further studies are needed to compare Propofol impacts with other anaesthetic drugs like ketamine, pentothal to prevent possible side effects in human.

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### ASSESSMENT OF NICKEL AND CHROMIUM CONCENTRATIONS IN BLACK KITE (*MILVUS MIGRANS*) TISSUES

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The present study was conducted to evaluate the concentrations of Ni and Cr in livers and muscles of 24 black kites (*Milvus migrans*) captured from Gujrat (34°17'26.05"N; 72° 9'39.23"E) and Kotla (32°51'7.63"N; 74° 4'23.57"E) of Punjab, Pakistan. The main objective of the study was to quantify Ni and Cr as well as providing indirect information regarding contamination of their habitats. The heavy metals analysis was performed by atomic absorption spectrophotometry. Overall the mean concentration of Ni and Cr were recorded higher in muscles than livers at both localities, furthermore both metals concentrations were recorded higher in the samples of Kotla Arab Ali Khan than Gujrat. T-test comparison revealed highly significant ( $p < 0.001$ ) relationship between Ni and Cr concentration in muscles Vs muscles and livers Vs muscles at both sampling localities, whereas correlation between muscles Vs muscles and livers Vs muscles were recorded strongly positive ( $r = 0.324$  and  $r = 0.599$ ) at Kotla while negative ( $r = -0.326$  and  $r = -0.271$ ) at Gujrat, respectively.

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**EFFECTS OF TRAMADOL ON HISTOPATHOLOGICAL AND BIOCHEMICAL PARAMETERS IN MICE (*MUS MUSCULUS*) MODEL**

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The present study was conducted to determine the histopathological and biochemical changes in liver due to injection of tramadol in albino mice (*Mus musculus*). For this purpose, forty albino mice (25-30gm) were divided into four groups, each group carried ten mice (three experimental and control group). Experimental group (B, C and D) was injected tramadol intramuscularly equal to 12.5 mg, 25mg and 50 mg/kg body weight/day respectively for fourteen days. Biochemical analysis indicated that the levels of serum aminotransferase (ALT, AST) significantly ( $P < 0.05$ ) increased than the control group. Similarly creatinine and blood urea nitrogen (BUN) were also increased significantly ( $P < 0.05$ ) in the experimental groups than control. The histopathological studies indicated the necrosis, vacuolization, central vein dilation, hemorrhage, cytolysis and complete cell membrane degeneration in hepatocytes in the treated groups. Therefore it is recommended that tramadol should be taken only with the prescription of doctor and self medication of this medicine may be hazardous.

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**MOLECULAR CHARACTERIZATION OF CHROMIUM AND ARSENIC RESISTANT BACTERIA FROM POLLUTED PAKISTANI SOILS**

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Microbial community is playing efficient role in the bioremediation of polluted environment. The present study deals with the isolation and characterization of chromium and arsenic resistant bacteria and their role in the bioremediation of metal polluted wastewater/soils. Arsenic and chromium resistant bacteria were isolated from soils from three industrially contaminated sites near the cities of Kasur, Sialkot and Sheikhupura, Punjab, Pakistan. Isolates with higher arsenic and chromium resistance were further characterized phylogenetically and physiologically. From contaminated soil samples, ten strains with high arsenic resistance and fifteen strains with high chromium resistance were characterized. The minimum inhibitory concentration to arsenic ranged from 60 to 260mM. These strains were also resistant to other metals including cadmium, cobalt, copper and zinc, as well as to antibiotics. The bacteria were identified by 16S rDNA sequencing. Phylogenetic analysis showed that the strains were related to *Aeromonas punctata*, *Enterobacter cloacae*, *Bacillus licheniformis*, *Klebsiella pneumoniae*, *Kocuria*

*palustris*, *Sinorhizobium* sp. and *Acinetobacter* sp., *B. licheniformis*, *Exiguobacterium* sp., *B. pumilus*, *Staphylococcus pasteurii*, *Cellulosimicrobium cellulans* and *B. cereus*. Strains of *E. cloacae* subsp. *cloacae*, *K. pneumonia* and *A. punctata* reduced arsenate more efficiently. Genes for the arsenite efflux pumps *arsB* and *ACR3*, required for arsenic resistance in other organisms were detected in these isolates. The extent of Cr (VI) reduction was also evaluated using Diphenyl-carbazide method and reduction of Cr (VI) was found to be increased in the presence of other heavy metals especially  $MnSO_4$ . The optimum temperature and pH for chromium reduction varied with different strains. It was also found that penicillin enhanced the reduction rate. The enzyme involved in chromium reduction had been isolated in crude form from these chromium resistant strains. Most numerous, cultured bacteria in arsenic contaminated Pakistani soils were members of the *Proteobacteria*, which is consistent with pyrosequencing results that showed *Proteobacteria* dominance in these soils. The presence of such indigenous bacteria that reduce chromate and arsenate may allow remediation of contaminated soils.

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**DETERMINATION OF HEAVY METALS CONCENTRATION IN WATER AND MUSCLES AND GILLS OF FRESH WATER FISH *TOR PUTITORA* AT INDUSTRIAL AREA IN RIVER BARANDU, DISTRICT BUNER, PAKISTAN**

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This study of determination of heavy (Fe, Cd, Pb, Mn) in water and tissues (muscles and gills) freshwater fish *Tor putitora* from River Barandu district Buner was conducted during April to November 2012. The heavy metals concentration was determined by using atomic absorption spectrometry. Marbles industries are situated along the entire river discharging enormous amount of effluents to the river. Three different sampling stations were selected of River Barandu (upstream, industrial site and downstream). Higher mean value of heavy (Fe, Cd, Pb, Mn) were recorded in the water and tissues (muscles and gills) at industrial site comparing to other sites. Trace metals contents were found increased significantly ( $P < 0.05$ ) from upstream toward industrial site and downstream. It was concluded from the present study that all the Trace metals were recorded comparatively higher at industrial site, while diluting at downstream but still was found higher from upstream. Such exceeding proves the presence of large quantities of heavy metals contents in effluents discharging from marble industries. It is recommended that marble industries should develop away from the water body and reclamation tank should develop to recycle the polluted water before discharging to the river.

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**TOXIC EFFECTS OF *ODONTOBUTHUS ODONTROUS* (ARACHNIDA: BUTHIDAE) VENOM IN ALBINO MICE**

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Present study was undertaken to evaluate the in-vivo toxic effects of *O. odontrous* venom on male albino mice. Scorpions were collected from undisturbed sandy areas of district Sargodha. We extracted the venom, prepared different concentrations and injected intraperitoneally into healthy albino mice. Toxic effects of the venom were studied on different blood parameters i.e., total leucocyte count (TLC), neutrophil count, RBC's count and platelet count, lymphocytes, eosinophils and monocytes. The venom of *O. odontrous* caused drastic change in blood physiology of treated animals. We observed a significant decrease in RBC's, platelets and lymphocytes of treated animals. However, treated animals showed marked increase in neutrophils, hemoglobin and TLC count. There was no difference in the count of monocytes and eosinophils between treated and control animals. It is concluded from the study that venom of *O. odontrous* is potentially harmful to mammals as it badly affects the blood physiology which may ultimately leads towards many disorders in the body.

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**GENOTOXIC POTENTIAL OF CYPERMETHRIN AND MALATHION PESTICIDE ON FISH (*OREOCHROMIS MOSUMBICUS*)**

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The micronucleus assay has been used to evaluate genotoxicity of many compounds in polluted marine ecosystems. The aim of this study is to verify the efficiency of the micronucleus assay in laboratory, using erythrocytes of the tilapia specie (*Oreochromis mosumbicus*) as genotoxicity biomarkers. Different doses of cypermethrin and malathion were injected intraperitoneally and specimen were sacrificed after 24 and 48 hours. Gill peripheral blood samples of tilapia were obtained and smear was made on glass slides. In order to investigate the frequencies of micronuclei and to assess the sensitivity of species, the results were compared with control samples. Our results revealed significant dose dependent increase in the frequencies of micronuclei in pesticide treated fish as compare to control.

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### **ESTIMATION OF INSECTICIDE DETOXIFYING ENZYMES IN MALATHION RESISTANT HOUSEFLIES FROM SARGODHA DISTRICT**

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The present study was designed to evaluate the role of Insecticides detoxifying enzymes (*i.e.*, Esterases, Glutathione S-transferases and Monooxygenases) in malathion resistant Houseflies. For the study flies were collected from three different localities *i.e.*, Rehman Pura, Sultan Colony and Chak No, 75-A, SB. Flies from all studied populations were found resistant to the tested concentrations of malathion. To measure the activities of Esterases, Glutathione S-transferases and Monooxygenases, methods of Asperen (1962), Habig *et al.* (1974) and Vulule *et al.* (1999) were followed respectively. The activities of  $\beta$  esterases among resistant flies were inhibited in all populations. However, the activities of GST and Monooxygenases were significantly higher among resistant flies compared to susceptible flies. We concluded from the study that higher level of GST and Monooxygenases in the malathion treated flies indicate the involvement of these enzymes in the malathion resistance.

### **ASSESSMENT OF HEAVY METALS IN THE ORGANS OF CATLA CATLA REARED UNDER DIFFERENT TREATED WASTEWATER DILLUTIONS AT WASA TREATMENT PLANT IN FAISALABAD, PAKISTAN**

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Accumulation of heavy metals in fish organs of *Catla catla* was assessed using atomic absorption spectrophotometer. Five concentrations of wastewater *i.e.* 20%, 40%, 60%, 80%, 100% were made and each aquarium was filled with respective concentration. One aquarium was kept as control and no wastewater was added to it. The test medium was renewed on weekly basis to maintain the desired wastewater dilution. Single breed of *Catla catla* were kept in respective aquaria maintaining a constant temperature (30°C) and pH (7.00). At the end of 90 days trial, the fish from each aquarium was dissected and tissue samples were digested in aqua regia for the analysis of heavy metals under atomic absorption spectrophotometer. The results indicated that the order of accumulation of heavy metals was Zn > Co > Cu > Ni > Cd. It was noticed that metal accumulation in various organs of fish vary significantly ranging from highest in liver to the lowest in muscles and fins *i.e.* metals accumulated in pattern: liver > kidney > gills > bones > skin >

muscles > fins. The amount of metal accumulation also depends on the concentration of wastewater made for the experimental trial. The maximum amount of heavy metals was accumulated in 100% waste water and the least amount of heavy metal was accumulated in control medium which was devoid of wastewater.

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**GROWTH AND METALS BIOACCUMULATION IN *CIRRHINA MRIGALA* DURING CHRONIC DUAL EXPOSURE OF WATERBORNE AND DIETARY METALS**

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Growth performance of 120-day old fish, *Cirrhina mrigala*, was monitored for 96 days, under dual exposure of waterborne and dietary copper, cadmium and zinc, separately, in glass aquaria with three replications for each treatment. Separate groups of fish were simultaneously exposed to sub-lethal dual toxicity of waterborne and dietary (1/3<sup>rd</sup> of LC<sub>50</sub> and LD<sub>50</sub>) copper, cadmium and zinc at constant water temperature, pH and hardness. The growth performance of fish was determined on fortnightly basis in terms of wet weight, fork and total length increments, condition factor, feed intake and feed conversion efficiency. The control (un-stressed) fish showed significantly better growth, condition factor and feed conversion efficiency than those stressed with copper, cadmium and zinc. Among treatments, zinc stress to the fish gave significantly better growth in terms of weight, fork and total length increments than that observed due to copper and cadmium treatments. Zinc stressed fish showed significantly better feed intake while it was low due to copper. Cadmium stress to the fish caused significantly better feed conversion efficiency, followed by that of zinc and copper treatments. However, condition factor of fish did not change significantly due to three treatments. During growth trials, fish liver accumulated significantly higher copper, cadmium and zinc concentrations of 49.36, 49.35 and 50.35  $\mu\text{g g}^{-1}$  while fish bones showed significantly lower amassing of 8.81, 8.67 and 9.27  $\mu\text{g g}^{-1}$ , respectively. The accumulation of zinc in fish body was significantly higher than copper and cadmium. However, the difference between copper and cadmium accumulation in fish body did not differ significantly.

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**EFFECT OF NEEM LEAVES TOXICITY ON THE GILL STRUCTURE, BLOOD CHEMISTRY AND ENZYME ACTIVITY OF *OREOCHROMIS MOSSAMBICUS***

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The experimental trail was conducted on *Oreochromis mossambicus* to assess

the Neem leaves toxicity on the gill structure, blood chemistry and Enzyme activity at control (No additives) , 0.30, 0.60, 0.90 1.20, 1.50 and 1.80ppm. in a large cement tank (1,000 litre capacity).. The leaves of Neem (*A. indica*) were collected, dried and grinded finely. The dried leaves were soaked in ratio of 50 gin two liter of water for 24Hours at room temperature. Then the mixture was filtered and the extract (25 g l-l of stock solution) was used immediately for the experiment at different dilutions. During the rearing (45 days) fish were fed on rice polish and water was changed daily. Above 0.90ppm concentration neem leaves caused gill degeneration, decreased in Hb (Hemoglobin), RBC (Red blood cell), MCV (Mean Cell Volume). During the study period, the hematological parameters including Hb, Hct (Hematocrit), RBC, MCV, MCH and MCHC (Mean Cell Hemoglobin Concentration) levels were significantly decreased in neem leaf extract exposed fish when compared to the control fish whereas MCH and MCHC count was increased. In biochemical study, elevated plasma glucose levels were noticed. The enzymes, activities were decreased significantly in intestine of treated fish compared to that of their control groups. The results of the present investigation suggest that Neem leaf extracts affects the hematological, biochemical and enzymological parameters of fish and alterations of these parameters can be useful in environmental bio monitoring of Neem based products in freshwater environment.

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#### **EFFECTS OF LAMBDA CYHALOTHRIN EXPOSURE ON SERUM LEVEL OF TESTOSTERONE IN SPRAY WORKERS**

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Keeping in view the health hazards and possible endocrine disrupting characteristics of pesticides present study was aimed to evaluate the toxic effects of *viz.* Pyrethroid (Lambda Cyhalothrin) insecticide with particular reference on male reproductive function in the spray workers. In this regard little research work was carried out and reported in Sindh, Pakistan. To this, serum level of circulating testosterone in agro field workers/ farmers was assessed. Forty spray workers (age group 20-45 years) were voluntarily participated in our study and exposed to Pyrethroid (Lambda Cyhalothrin 2.5 EC) insecticide through spraying on crops. Participants were assessed through questionnaire regarding their occupational history, exposure to pesticides and health condition. Thereafter blood samples before and after spray were collected for the assessment of serum testosterone. The hormonal assessment was made by procedure electrochemiluminescence immunoassay (ECLIA) at Research and Diagnostic laboratory, Liaquat University of Medical and Health Sciences, Hyderabad, Pakistan. After exposure to lambda cyhalothrin 60% of the total spray workers had significant change in the serum level of testosterone ( $4.77 \pm 0.99 \text{ ng./ ml.}$   $P < 0.05$ ) as compared to before spraying serum level of testosterone  $5.77 \pm 0.97 \text{ ng./ ml.}$  Whereas in 40% of the total participants the change was insignificant. Results of present study reveal that lambda cyhalothrin

insecticide caused disruption on the serum testosterone. Furthermore we suggest that, indiscriminate usage of pesticides and inappropriate protective measures may impair the reproductive function in spray workers. Further research work to evaluate the extent and potential toxic effects of pesticides is recommended.

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#### ASSESSMENT OF PERSISTANT ORGANIC POLLUTANTS ON COTTON-PICKERS WOMEN IN KHANEWAL DISTRICT, PAKISTAN

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The cotton picker women community in Southern Punjab is highly vulnerable to the use of pesticides for crop protection. About 35 female cotton pickers were sampled to highlight the impacts of pesticides on their body fluids such as blood and milk in District Khaniwal. The present study was aimed to determine the present status of pesticides in blood and milk of cotton field worker and highlight the impacts of residual pesticides on the women health. Mean concentration of HCH (a-HCH  $3.216 \pm 4.932$  mg/mL; b-HCH  $3.862 \pm 9.221$  mg/mL; e-HCH  $6.736 \pm 15.322$  mg/mL; d-HCH  $5.249 \pm 9.851$  mg/mL, r-HCH  $120.193 \pm 715.723$  mg/mL and HCB  $2.805 \pm 9.056$  mg/mL), b-endosulfan ( $3.671 \pm 16.122$  mg/mL), op-DDD ( $17.593 \pm 38.754$  mg/mL), pp-DDD ( $12.208 \pm 14.762$  mg/mL), op-DDT ( $8.902 \pm 14.172$  mg/mL), pp-DDT ( $14.349 \pm 22.391$  mg/mL), PP-DDE ( $20.278 \pm 37.032$  mg/mL), op-DDE ( $10.442 \pm 21.969$  mg/mL) and Heptachlore ( $1.330 \pm 1.761$  mg/mL) were measured in milk samples. Mean concentration of HCH (a-HCH  $1.042 \pm 4.511$  mg/mL; b-HCH  $11.596 \pm 23.539$  mg/mL; e-HCH  $2.920 \pm 20.751$  mg/mL; d-HCH  $17.217 \pm 9.355$  mg/mL r-HCH  $35.353 \pm 1.159$  mg/mL and HCB  $13.634 \pm 2.145$  mg/mL), b-endosulfan ( $7.520 \pm 5.154$  mg/mL), Total content of DDE ( $730.037$  mg/mL), Heptachlore ( $7.938 \pm 1.106$  mg/mL), op-DDD ( $3.469 \pm 11.066$ ) pp-DDD ( $37.834 \pm 22.535$ ), op-DDT ( $25.788 \pm 40.038$  mg/mL), pp-DDT ( $29.748 \pm 36.088$  mg/mL), op-DDE ( $51.042 \pm 25.028$  mg/mL) and pp-DDE ( $22.689 \pm 81.114$  mg/mL) were measured in blood serum samples. The level of different species of pesticides in blood and milk varies significantly. The level of pesticides in the milk and blood of cotton picker women in Khanewal District were higher than the international permissible limits. Results of present study will be helpful in women health protection and development. This study also recommends some stringent measures related to pesticide use addressing the women health and environmental issues.

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**MOLECULAR AND BIOCHEMICAL ASSESSMENT OF ALONE OR  
COMBINED EXPOSURE OF COPPER AND COBALT IN A LOCAL SPECIES  
OF CARP (*CYPRINUS CARPIO*)**

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Cobalt and copper are those heavy metals that act as cofactor for various enzymes. They also act as micronutrients and are therefore physiologically relevant in fishes. Contrary to this, concentration of these metal elements in water bodies greater than normal due to anthropogenic activities can be harmful especially in case of fish making fish at one end toxic and non-edible for consumption but on the other as good bio indicators of toxicity. The present study was conducted to evaluate impact on biochemical and molecular aspects of car body tissues following 96 hrs exposures to cobalt (250 mg/l as cobalt chloride) and copper (0.25mg/l as copper sulphate) in combined and as well alone treatments. Five tissues including kidney, muscle, skin liver and gills of fish *C. carpio* were analyzed for atomic absorption spectrophotometry for estimation of Mn, Pb, Zn, Ni, Cr, Co, Cu and Fe. Blood samples were analyzed for DNA damage. Results were compared statistically followed by post-hoc tests. Pearson's correlation identified correlations in different metals. In the combined Co+Cu treatment group; in kidney tissue, Mn ( $P<0.006$ ), Ni ( $P<0.031$ ), Cr ( $P<0.037$ ), and Cu ( $P<0.026$ ) concentration decreased significantly while Co ( $P<0.006$ ) concentration increased significantly. No effect was found on Fe and Pb. In muscle tissue, Pb concentration ( $P<0.004$ ) showed significant decrease while Co concentration ( $P<0.013$ ) increased in muscle tissue. In the skin, only Mn ( $P<0.025$ ) concentration showed significant increase. Liver showed elevation in Zn ( $P<0.005$ ), Cu ( $P<0.001$ ) and Fe ( $P<0.001$ ) concentrations while Ni ( $P<0.001$ ) levels decreased. In gills, Zn concentration ( $P<0.003$ ) decreased significantly while Cu ( $P<0.001$ ) concentration was increased when compared with cobalt alone and control. Fe concentration ( $P<0.0001$ ) was increased when compared with control but Fe showed decreased concentration ( $P<0.001$ ) when compared with Cu alone. In the Cu alone treatment group Cu concentration ( $P<0.048$ ) showed decrease in skin tissue while, Cu ( $P<0.0001$ ) and Fe ( $P<0.0001$ ) concentrations increased in liver tissue. On the other hand, Zn ( $P<0.003$ ) concentration decreased in gills while Cu concentration increased ( $P<0.0001$ ) when compared with control. In the Co alone treatment group Ni ( $P<0.0001$ ) and Cu ( $P<0.0001$ ) concentrations increased in liver tissue and Fe concentration ( $P<0.0001$ ) increased in gills, while Co concentration ( $P<0.0001$ ) decreased as compared to Cu alone. In muscle tissue, Pb ( $P<0.004$ ) concentration decreased while in gills Fe concentration decreased ( $P<0.0001$ ) as compared to Cu alone treatment group. In the combined treatment group negative and positive correlation among different metal elements were observed in kidney, skin and liver tissue. In the Cu alone group maximum correlations were observed in the kidney and gills. In Co alone group, negative and positive correlation were observed in kidney, muscle, skin and liver tissues. A greater genotoxic effect was demonstrated by Cu alone

treatment and also by Co+Cu combined treatment while slighter DNA damage took place in the Co alone treatment. The study concluded that copper is relatively more toxic for this fish species in alone as well as in combined treatment states. On the other hand cobalt appears to have countered the copper toxicity. The study suggested that cobalt can be used as an antidote towards copper toxicity. Further work is however required to know exactly by which mechanism cobalt is able to produce its attenuating effect.

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**ASSESSMENT OF PARATHYROID AND ADRENAL FUNCTIONS  
FOLLOWING TREATMENT WITH SELENIUM AND ASCORBIC ACID IN  
HEXAVALENT CHROMIUM TREATED LABORATORY RATS**

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In Pakistan, heavy metal content is on consistent rise in the environment due to industrialization and poor sewerage system. Humans are continuously being exposed to toxic, carcinogenic and teratogenic effects of hexavalent chromium due to its occupational exposure and accumulation in sediments, water and food chain. The oxidative stress caused by chromium results in lipid peroxidation and generation of reactive oxygen species (ROS). The present study investigated if toxicity induced by hexavalent chromium to adrenal and parathyroid glands is counteracted by selenium and ascorbic acid, two well-known antioxidants. Thirty Sprague-Dawley rats were divided into five groups; control (saline treated) and four experimental groups, group II (only chromium 2 mg/kg <sub>b.w.</sub>), group III (chromium and ascorbic acid (20 mg/kg <sub>b.w.</sub>), group IV (chromium and selenium (1 mg/kg <sub>b.w.</sub>), and group V (chromium, ascorbic acid and selenium). The cortisol levels were determined through an ELISA kit (Amgenix, INC. USA). One way ANOVA was applied to compare serum cortisol level among groups. Standard histology was performed to study cellular alteration in the glands. Chromium administration led to an elevation of serum cortisol as compared to the control. Both antioxidants when given alone showed decline of cortisol concentration as compared to chromium only treated group. Combined treatment with the two antioxidants kept serum cortisol levels high as compared to all groups. Although there occurred some difference in serum cortisol concentration among different groups, the results were not statistically significant ( $P < 0.282$ ). The chromium alone treated group showed cellular alterations in adrenal and parathyroid gland. Some degree of mononuclear infiltration, enlarged cellular spaces and tissue thickenings in the adrenal gland. The parathyroid gland appeared slightly damaged along with cellular spaces and tissue thickenings. Gross abnormalities were however not observed at this dose of chromium. Other groups showed no cellular abnormalities when compared with the control group. It is concluded that even low dose of chromium is capable of inducing toxicity and tissue damage. While the antioxidant, ascorbic acid and selenium provides adequate oxidative protection against the hexavalent

chromium further investigations are however required in order to have in depth understanding of chromium toxicity.

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**UPTAKE OF HEAVY METALS (COPPER AND CHROMIUM) BY  
PARAMECIUM SPECIES ISOLATED FROM WASTE WATER**

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Three identified species of *Paramecium*, *Paramecium multimicronucleatum*, *Paramecium caudatum* and *Paramecium tetraurelia* were obtained from the cell and molecular biology lab department of Zoology, University of the Punjab, Lahore. The resistance of *Paramecium* was checked against two heavy metals, copper and chromium. The metal ions slowed down the growth of the ciliates as compared to the culture grown without metal stress. Particular metal ion was added at the concentration of 1.0 µg/mL every day for eight days. The number of cells obtained after copper treatment in cultures of *Paramecium multimicronucleatum*, *Paramecium caudatum* and *Paramecium tetraurelia* are 3866, 1666 and 3066 as compared to their control cultures 4332, 5460 and 3200 respectively. The number of cells obtained after chromium treatment in cultures of *Paramecium multimicronucleatum*, *Paramecium caudatum* and *Paramecium tetraurelia* are 2132, 1233 and 1133 as compared to their control cultures 4332, 5460 and 3200 respectively. Ciliates have shown remarkable ability to pick up metal ions from the culture medium. *Paramecium multimicronucleatum* reduced 87% of copper and 95% of chromium from the culture medium after 96 hours of incubation. *Paramecium caudatum* reduced 77% of copper and 96% of chromium from the culture medium after 96 hours of incubation. *Paramecium tetraurelia* reduced 71% of copper and 81% of chromium from the culture medium after 96 hours of incubation. From these results, it can be concluded that the ability of ciliates to take up multiple heavy metals from the medium could be exploited for metal detoxification and environmental clean-up operations.

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**CADMIUM INDUCED TERATOGENIC EFFECTS IN DEVELOPING *MUS MUSCULUS***

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This study was designed to evaluate the teratogenic and embryotoxic effects of cadmium on developing mice. The pregnant mice were exposed to dose groups 0.00, 3.13, 6.25, 12.5 and 28.0 µg/g B.W on days 6 to 12 of gestation daily. Fetuses recovered on day 18 of gestation, were analyzed on morphological, morphometric, histological and skeletal basis. Morphological studies showed the abnormalities such as distorted axis,

cryptophthalmia, anophthalmia, open eyelids, micromelia, phocomelia, agnathia, hydrocephaly, angular tail and subcutaneous hemorrhages. Morphometric analysis indicated a significant ( $p < 0.05$  to  $p < 0.001$ ) reduction in fetal body weight, crown rump length, head circumference, eye circumference, forelimb and hind limb lengths and tail size against controls. Histological observations showed brain defects, dilation in the 3<sup>rd</sup> and 4<sup>th</sup> ventricles (internal hydrocephaly), absence of 3<sup>rd</sup> and 4<sup>th</sup> ventricles (internal microcephaly) and under developed lateral part of cerebellum. Lungs defects included necrosis and underdeveloped lungs lobes which heart anomalies included microcardia, hypoparicardium and necrosis in atrium and ventricles. Sections through liver shows necrosis and apoptosis and misshapen lobe. The transverse sections through kidney also showed necrosis and in some cases right kidney was missing. Skeleton studies showed partially and completely unossified bones as compare to control. It is concluded that the concentrations used in present study proved teratogenic in mice fetuses.

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#### **EMBRYOTOXIC EFFECTS OF DELTAMETHRIN IN DEVELOPING MURINE EMBRYOS**

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Present study was focused to evaluate the embryo toxic effects of deltamethrin in developing murine embryos. Different concentrations of deltamethrin viz. 19.36, 9.7 and 4.8  $\mu\text{g/g}$  BW were given orally to pregnant mice on the day 6 and 9 of gestation. The female mice were scarified on the day 18 of gestation. The morphological anomalies include open eyelids, under developed eyes, hyperextension of forelimb, ectomelia, micromelia, hydrocephaly, sacral hygroma, anophthalmia, microphthalmia, reduced spinal length drooping wrist, doomed back, short and kinked tail and resorbed uterus. The brain and body haemorrhage were also observed in the treated groups. The morphometric analysis indicates significance reduction in body weight, crown rump length, brain and eye circumference, length of limbs and tail as compared to control and vehicle control.

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#### **HEXAVALENT CHROMIUM CAUSES FETAL DEFECTS AFTER EXPOSURE TO PREGNANT MICE**

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Chromium level is shown to be high in different parts of the world. Hexavalent Chromium [Cr(VI)] besides general toxicity, is involved in developmental toxicity. The

work was done to check teratogenic effects of different doses of Cr(VI), given to pregnant mice. LD<sub>50</sub> of Cr(VI), was calculated as 88 mg/kg of B.W in pregnant female mice. Among 5 groups of pregnant females, three experimental groups were forced fed with 50%, 25% and 12.5% of LD<sub>50</sub> of Cr(VI) respectively, along with one control and one vehicle control group. Fetuses were recovered and fixed for morphometric and morphological studies. Experimental groups showed variable degree of occurrence of fetal abnormalities, along with reduction in many external organ sizes parallel to increase in dose concentration, as compared to control and vehicle control. Exencephaly, Omphalocele, hygroma and many limb abnormalities were recorded. It is shown that Cr(VI) causes teratogenic effects in gross anatomy in mice embryos.

**STUDIES ON TOXIC EFFECT OF THREE SELECTED HEAVY METALS  
(LEAD, CHROMIUM, CADMIUM) ON POULTRY BIRD *GALLUS  
DOMESTICUS***

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TABASSUM AND MUHAMMAD TABISH  
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The objective of the study was to investigate the toxic effect of heavy metals Lead, Chromium, and Cadmium on poultry bird *Gallus domesticus* which are widely consumed from local market of Karachi. The level of blood parameters were decreased with Pd where as Chromium and Cadmium showed increased level of blood parameters. RBC level was decreased with Cd and Pb where as WBC level was decreased with Pb and Cr Chromium. Lead also decreased the life span of RBC. The strong dose of lead showed mean concentration of Hb was 13.42 gm/dl, R.B.C 3.33 E/L, M.C.V 160.30 fl, M.C.H 40.74 pg, M.C.H.C 29.18 %, and W.B.C 189 xe/I respectively where as low dose showed mean concentration of Hb as 12.52 gm/dl, R.B.C 3.18 E/L, MCV 155.72 fl, M.C.H 39.44 pg, M.C.H.C 27.99 % and W.B.C is 207.50 xe/l respectively. The strong dose of cadmium showed mean concentration of Hb was 14.87 gm/dl, R.B.C 3.19 E/L, M.C.V 129.69 fl, M.C.H 48.38 pg, M.C.H.C 37.56 %, W.B.C 135.24 xe/I where as low dose showed mean concentration of Hb was 14.21 gm/dl, R.B.C 3.11 E/L, MCV 127.51 fl, M.C.H 47.54 pg, M.C.H.C 36.70 % and W.B.C 145.75 xe/1. The strong dose of chromium showed mean concentration of Hb as 9.35 gm/dl, R.B.C 2.03 E/L, M.C.V 142.40 fl, M.C.H 37.20 pg, M.C.H.C 23.95 % and W.B.C 235.02 xe/I where as low dose showed mean concentration of Hb as 10.40 gm/dl, R.B.C 2.37 E/L, MCV 148.02 fl, M.C.H 39.42 pg, M.C.H.C 25.60 % and W.B.C 207.31 xe/l. Histopathology, Induced high dose of heavy metals (Pb, Cr & Cd) showed the abnormalities of cells size and function, damage of cells and tissue of liver, kidney, intestine and brain. The experiment was carried on selected bird *Gallus domesticus* (chicks) which was obtained from the local market of Karachi. Bird was kept in controlled condition for 3 to 5 days before experimental work, and then birds were divided into three groups: (a) control; (b) Weak

dose (10 µg/body weight) and c) Strong dose 20 µg body weight). Chromium, Cadmium, and lead were used in the form of salts, chromium sulphate (CrSo<sub>4</sub>), Cadmium Chloride (CdCl<sub>2</sub>), and lead nitrate (PbNo<sub>3</sub>). These were used as a weak dose (10 µg/body weight) and strong dose (20 µg/body weight). (Dacie and Lewis 1977 methods were applied for estimation of following parameters: Erythrocytes count (RBC), Leukocytes count (WBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC), and Mean corpuscular haemoglobin (MCH).

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### **IMPACT OF SUB-LETHAL CONCENTRATIONS OF COBALT ON THE GROWTH PERFORMANCE OF *LABEO ROHITA***

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This project was designed to study the effect of sub-lethal concentrations of water-borne and dietary cobalt on the growth performance of *Labeo rohita* during chronic exposure. The experiment was conducted in glass aquaria at constant water temperature, pH and total hardness of 30°C, 7 and 200 mg L<sup>-1</sup>, respectively. The control fish were grown under cobalt free water and fed with normal (metal free) diets. The fish were grown for 12 weeks in a water medium containing sub-lethal concentration of cobalt and fed with a diet, to satiation, containing sub-lethal (1/3<sup>rd</sup> of LD<sub>50</sub>) concentration of cobalt. The water pH, total hardness, calcium, magnesium, sodium, potassium, dissolved oxygen, electrical conductivity, carbon dioxide, temperature and total ammonia of the test mediums and control were monitored on daily basis. The growth of fish was determined in terms of wet weights, fork and total lengths (gains/decrease), length-weight relationships and condition factor of fish during the period of this investigation. The data obtained from this experiment was analyzed by using Factorial Experiment (RCBD) and Tukey's/Student-Newman-Keul tests. Regression and correlation analyses were performed to find-out relationships among various parameters under study. It was found that chronic sub-lethal mixed exposure of water-borne and dietary cobalt to the fish, *Labeo rohita* caused significant impact on its growth performance in terms of wet weights gain, fork and total lengths. However, control fish had attained significantly higher growth rate than that of metal exposed fish. All the three age groups of fish showed significantly better feed intakes and feed conversion ratios for control than treated fish. The treated fish exhibited low condition factor and growth rate as compared to control fish. Exposure of water-borne and dietary cobalt to the fish resulted in increased oxygen consumption by the fish with an escalated ammonia excretion by the fish.

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**HISTOLOGICAL AND BIOCHEMICAL STUDY OF LIVER OF SILVER CARP  
(*HYPOPHthalmICTHYES MOLITRIX*) AFTER ACUTE EXPOSURE TO  
DELTAMETHRIN**

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Deltamethrin, a broad spectrum synthetic pyrethroid pesticide is considered less toxic as compared to organochlorine and organophosphate pesticides due to its easy degradation in soil. It is more toxic to aquatic than terrestrial organisms. Deltamethrin was chosen to study the biochemical and histological alteration in liver of silver carp. LC<sub>50</sub> was determined before experiment. LC<sub>50</sub> was found to be 1.6/-µg/L for 96 hours. For the experimental purpose fish were divided into 4 groups A, (control) E, C & D (experimental). In each group 6 fish were introduced into plastic tubs with 40 liter water with continuous aeration. Test fishes were exposed to 25%, 50% and 75% of Lc50 for acute exposure. After 96 hours sampling of blood and liver was done. Histology of liver and biochemical tests for two liver enzymes Alanine aminotransferase (ALT), and Aspartate Amino-transferase (AST) were performed. Histology of liver shown necrosis, nuclear pycnosis, hypertrophy of hepatocytes, vacuolation and congestion of blood vessels indicating damage due to acute exposure of pesticide. This result was also supported by the significant increase in hepatic enzymes AST and AL T levels in blood plasma of exposed fish as compared to control group. These results suggested that deltamethrin is highly toxic for fish.

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**PROTECTIVE ROLE OF SWEET ORANGE (*CITRUS SINENSIS*) JUICE ON  
THE CYPERMETHRIN INDUCED HISTOPATHOLOGICAL CHANGES IN  
PREGNANT MICE LIVER AND KIDNEYS**

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Histopathological changes of cypermethrin (CYM) exposure in pregnant mice liver and kidneys against the protective role of concurrent "Sweet orange" (*Citrus × sinensis*) juice feeding were investigated. Four groups of 10 pregnant dams were designated as control (C); cypermethrin (CYM); Sweet orange juice (SOJ) and SOJ+CYM groups. While, SOJ and SOJ+CYM groups were provided orange juice instead of drinking water (*ad-lib*) through out the study period; the CYM and SOJ+CYM groups received 50mg kg<sup>-1</sup> CYM on gestation days (GD) 6, 9 and 12 by gavage. Organs (liver and kidneys) were obtained from the euthanized dams on GD18 and processed for histopathological study. Disfigured nuclei, cytoplasmic vacuolations and resultant

necrosis of hepatocytes were seen in CYM group. The micrometric data indicated that the mean cross-sectional area (CSA) of hepatocytes for C, CYM, SOJ and SOJ + CYM treated groups was  $133\mu^2$ ,  $220\mu^2$ ,  $135\mu^2$  and  $192\mu^2$  respectively. The pathological changes seen in the mice kidney in CYM group include obliterations of the glomeruli and associated tubules with infiltration of the macrophages and fibrosis in the cortical parts of the kidneys. Statistical analysis of the micrometric data showed significant ( $P<0.05$ ) increase in mean CSA ( $1001\mu^2$ ,  $1886\mu^2$ ,  $1127\mu^2$  and  $1437\mu^2$  for C, CYM, SOJ and SOJ+CYM respectively) of the glomeruli with a simultaneous reduction in mean CSA of proximal tubules ( $962\mu^2$ ,  $588\mu^2$ ,  $900\mu^2$  and  $693\mu^2$  for C, CYM, SOJ and SOJ+CYM respectively) and mean thickness of the tubular ciliated brush border ( $6.69\mu^2$ ,  $3.68\mu^2$ ,  $7.43\mu^2$  and  $5.48\mu^2$  for C, CYM, SOJ and SOJ+CYM respectively). Along with the micrometric curative indications in these parameters; the obvious histological signs recovery from the pathological indications were observed in SOJ+CYM group. These findings indicate curative potentials of frequent use of *Citrus × sinensis* fruit juice in cypermethrin intoxicated pregnant mice.

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**APPRAISAL OF GROUND WATER QUALITY AND ITS SUITABILITY FOR  
DRINKING USES IN SOME SELECTED AREAS OF TEHSIL SAMAHNI,  
DISTRICT BHIMBER, AZAD JAMMU & KASHMIR**

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Present study was planned to obtain an opinion that either the ground water of Tehsil Samahni is safe for drinking or not. A total of 7 water samples were collected from Tehsil Samahni to analyze for various physicochemical and biological parameters *i.e.* namely temperature, pH, turbidity, color, odor, taste, Electric Conductivity (EC), Total Dissolved Solids (TDS), Total Hardness (Calcium + Magnesium), Chloride, Arsenic, Nitrite and *Escherichia coli*. Results revealed that ground water of Tehsil Samahni was polluted with pathogenic micro organisms like *E.coli*. Besides it, values of some physicochemical water quality determining parameters were also found beyond the recommended limits suggested by World Health Organization (WHO) *i.e.* Chloride ion concentration was below the prescribed limits. It has been proven that consumption of unsafe drinking water is one of the major causes of prevalence of water born diseases like diarrhea, typhoid fever and malaria etc. in the study area. It is affirmed that ground water of Tehsil Samahni is not suitable for drinking without treatment.

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**DETERMINATION OF 96-HR LC<sub>50</sub> AND LETHAL CONCENTRATIONS OF BIFENTHRIN+CHLORPYRIFOS MIXTURE FOR *Oreochromis niloticus***

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Effluents from industries and runoff from agricultural areas contain pesticides that can accumulate and persist in aquatic environment, posing a serious threat to aquatic biota. The aim of this study was to determine the 96-hr LC<sub>50</sub> and lethal concentrations of bifenthrin+chlorpyrifos mixture for *Oreochromis niloticus* (Nile tilapia). Acute toxicity tests were performed by exposing the fish to various concentrations of pesticide mixture (bifenthrin+chlorpyrifos) by using static bioassay at constant water temperature (30°C), total hardness (225mgL<sup>-1</sup>) and pH (7.75). The observations on fish mortality were recorded during 96-hr duration for each test concentration. The data obtained from the acute toxicity tests were evaluated through Probit analysis method for the determination of 96-hr LC<sub>50</sub> and lethal concentrations (with 95% confidence interval) of bifenthrin+chlorpyrifos mixture for *Oreochromis niloticus*. Regression and correlation analyses were also performed to find-out statistical relationships among various parameters defined for this experiment. The mean 96-hr LC<sub>50</sub> and lethal concentration values of bifenthrin+chlorpyrifos mixture for *Oreochromis niloticus* were computed as 10.85±0.20 and 16.99±0.22 µgL<sup>-1</sup>, respectively. Study on physico-chemistry of test media revealed that total ammonia and carbon dioxide showed positively significant relationships with the concentrations of bifenthrin+chlorpyrifos mixture while dissolved oxygen contents exhibited significantly inverse relationship.

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**ASSESSMENT OF GENOTOXIC POTENTIAL OF ZINC FOR FRESHWATER FISH SPECIES**

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Laboratory tests were conducted to determine the genotoxic effects of zinc on four freshwater fish species. For this purpose fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Catla catla* were exposed to different sub-lethal concentrations (17, 25, 33 and 50% of 96-hr LC<sub>50</sub>) of zinc for a period of 30 days at constant water temperature (30°C), pH (7.5) and hardness (300 mgL<sup>-1</sup>). During this period fish were fed to satiation on feed (34% DP and 3.00Kcal/g DE) twice daily. Genotoxic effects of zinc were determined in-terms of percentage of damaged cells,

genetic damage index (GDI) and cumulative tail length of comets (CTL). Regarding overall performance of four fish species towards induction of DNA damaged cells, under zinc exposure, *Cirrhina mrigala* showed significantly higher frequency of damaged cells, followed by that of *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla*. However, *Labeo rohita* and *Ctenopharyngodon idella* showed statistically similar frequency of damaged cells. Zinc exposure to four fish species caused significantly variable GDI values to the erythrocytes at various concentrations. The mean GDI was significantly higher in *Cirrhina mrigala*, followed by that of *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla*. The mean cumulative tail lengths of comet, induced by various concentrations of zinc, in the erythrocytes of four fish species, varied significantly with the highest mean tail length of  $136.93 \pm 104.63 \mu\text{m}$  observed in *Cirrhina mrigala* while it was significantly lowest ( $69.84 \pm 52.07 \mu\text{m}$ ) for *Ctenopharyngodon idella*. Therefore, genotoxic effects of zinc were more pronounced in *Cirrhina mrigala* than *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla*.

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**ASSESSMENT OF HEAVY METALS (Fe, Mn, Cu, Zn, Ni, Pb) IN *THUNNUS TONGGOL* (BLEEKER, 1851) FISH FROM KARACHI FISH HARBOUR, KARACHI, PAKISTAN**

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Total (300) *Thunnus tonggol* fishes were collected seasonally for analysis of heavy metals during August, 2006 to December, 2011 from Karachi Fish Harbour. Concentration of heavy metals (Fe, Mn, Cu, Zn, Ni, Pb) determined in the muscle, liver, kidney and gills of *T. tonggol* fish. The highest mean length ( $66.0 \pm 7.472$  cm) and weight ( $3050 \pm 364.583$  gm) were measured in (2008-2009, 2009-2010). The maximum mean concentration of Fe ( $618.09 \pm 170.79$   $\mu\text{g/g}$  d.w), Mn ( $8.03 \pm 1.27$   $\mu\text{g/g}$  d.w), Cu ( $80.15 \pm 19.45$   $\mu\text{g/g}$  d.w), Zn ( $23.46 \pm 9.27$   $\mu\text{g/g}$  d.w), Ni ( $1.33 \pm 0.42$   $\mu\text{g/g}$  d.w), Pb ( $0.71 \pm 0.23$   $\mu\text{g/g}$  d.w) were determined in liver. Samples were analyzed by (AAS-700) Atomic Absorption Spectrophotometer. Our results indicated that all heavy metals were found highest in liver then muscles, kidney, gills and gonads. Metal concentration in the edible parts of fish was assed for human uses according to provisional tolerable weekly intake (PTWI) and provisional tolerable daily intake (PTDI). The estimated values of heavy metals in fish muscles were below and highest the established values recommended by (FAO/WHO 2004 as well as Fourteenth Schedule of Malaysian Food Regulations 1985). Therefore, it can be generalized that muscles of examined fish should pose health problems for consumers.

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### DIAGNOSTIC APPRAISAL OF INDUCED HEAVY METALS' TOXICITY IN PIGEONS

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In the wake of prolific urbanization and industrialization, and growing threats of toxic metals in our environment, it is pertinent to gauge the potential effects of heavy metals in biological systems. Therefore, the study deals about the heavy metals induced toxicity in pigeons by oral administration of one fifth of acute lethal dose (ALD<sub>50</sub>) of copper, zinc, arsenic and lead @ 5 mg/kg body weight, packed in gelatin capsules offered daily for twenty one days. The experiment was designed for two sets of trials: including 10 days in first phase and 20 days long trial in subsequent phase. The clinical signs of induced toxicity surfaced after two week post-exposure. These included gastrointestinal disturbances, lethargy, depression, weakness, anorexia, vomiting, polyuria, diarrhea, ataxia, head drooping, blindness, circling due to imbalance, paresis, paralysis, convulsions, polydipsia, feather picking, pruritus, weight loss, dyspnea, and renal insufficiency with 100% mortality in all the birds. Toxicity also induced severe degenerative changes and necrotic lesions in most tissue organs. Moreover, we observed pneumonia, hyperplastic goiter and lesions in skin of experimental birds. On account of toxicological findings, liver contained the largest residues of heavy metals followed by kidneys, body muscles and the lowest in brain. The intensity of toxicological changes was proportional to the accumulated amount of heavy metals in tissues organs. The spectrophotometric analysis of heavy metal treated organs was carried out to determine the amount of metals being bio-accumulated. In case of body weight of all treated birds, there was no significant difference ( $P>0.05$ ) with that of control group. However, there was a significant difference ( $P=0.002$ ) in liver tissues under 10 days, as well as, 20 days long ( $P=0.000$ ) experimental groups. Interestingly, there was a non-significant difference ( $P=0.39$ ) in Kidney tissues under 10 days experimental conditions, whereas, significant ( $P=0.005$ ) in 20 days long trial. Furthermore, we observed least significant difference ( $P=0.043$ ) in muscle tissues under 10 days and 20 days long ( $P=0.011$ ) trials. Similarly, there was least significant difference ( $P=0.038$ ) in brain tissues under 10 days trial and non significant ( $P=0.325$ ) under 20 days trial.

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## 9. LIVESTOCK

### **BLOOD BIOCHEMICAL PROFILE OF BROILERS, REARED UNDER CAGE-EXCHANGE-FLOOR REARING SYSTEM SUPPLEMENTED WITH DIFFERENT LEVELS OF MANNAN OLIGOSACCHARIDES (MOS)**

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A total of 240 day-old broiler chicks were reared under cage-exchange-floor rearing systems, ie. Floor-Cage and Cage-Floor supplemented with 4 levels of MOS (control, 400g/ton, 600g/ton, 800g/ton) after dividing the chicks randomly and equally in 2 major groups of 120 chicks. Blood sample were collected at 42 days of age after slaughtering two birds from each replicate. The data thus obtained were analyzed using Completely Randomized Design (CRD) and means were compared through Duncan Multiple Range test using SAS 9.1. On the basis of rearing systems, birds reared under Cage-Floor supplemented at 600g/ton of feed showed significantly ( $P<0.05$ ) increased blood glucose, cholesterol, creatinine and urea as compared to other treatments.

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### **SOME PRODUCTION PERFORMANCE AND EGG QUALITY TRAITS OF NAKED-NECK AND INDIGENOUS ASEEL CHICKEN OF PAKISTAN**

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The present study was conducted to evaluate production performance and egg quality of four different varieties of native Aseel chicken in comparison with naked neck. A total of 105 adult chickens, 21 birds from each of four varieties of Aseel namely Lakha, Mushki, Peshawari and Mianwali, and 21 birds from naked neck, were maintained separately. The data of daily egg production, daily egg weight and weekly egg quality were calculated for up to ten weeks and analyzed under Completely Randomized Design (CRD) through SAS 9.1 software. Comparison among treatment means were made through Duncan's Multiple Range (DMR) test. The results showed that egg production of naked neck (47.42) is significantly higher ( $P<0.05$ ) than that of Mushki (34.08), Lakha

(31.43), Mianwali (29.59) and Pesahwari (28.7). Egg weight of naked neck (57.52) and Peshwari (55.65) is significantly higher ( $P<0.05$ ) than lakha (54.03), Mushki (53.7) and Mianwali (51.62). Regarding egg quality traits, shell % of Peshawari (13.57) and Naked Neck (13.16) is significantly higher ( $P<0.05$ ) than that of Lakha (11.89), Mushki (10.19) and Mianwali (9.36). Similarly Haugh Unit Score of Naked neck (82.76) and Peshawari (81.95) is significantly higher ( $P<0.05$ ) than other varieties of Aseel. Albumen % was significantly higher in Mushki (61.83) variety while yolk % was significantly higher in Mianwali (59.36) variety of Aseel whereas yolk index showed non-significant ( $P>0.05$ ) difference in naked neck and all varieties of Aseel.

#### **EFFECT OF DIFFERENT WATER pH LEVELS ON GROWTH PERFORMANCE OF BROILERS**

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A study was conducted to evaluate the optimum level of water PH for the best broiler performance. 360 birds were tested at three different levels of water PH (4.4, 5.4, 6.4) using 2 organic acids formic acid (Soft acid as brand name) and other is Sodium hydrogen sulphate. Weekly data on feed intake, body weight, FCR, water intake and mortality were recorded. Statistical analysis showed that the birds reared at 6.4 pH of water for 24 hours, showed better results regarding feed conversion ratio (F.C.R), weight gain, mortality ratio and showed good tolerance to the bacterial as well as viral infections. Too low PH of water like 4.4 and 5.4 respectively showed comparatively lesser performance. Water intake also vary on variable water pH. Maximum water intake was also recorded at 6.4 pH as compared to others.

#### **GROWTH PERFORMANCE OF BROILERS, REARED ON 4-DIFFERENT REARING SYSTEMS**

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A total of 240 day-old broiler chicks were reared on 4 different rearing systems, i.e. Floor, Cage, Floor/ Cage and Cage/ Floor, after dividing the chicks randomly and

equally in 4 major groups of 60 chicks. Weekly data on body weight, feed intake, FCR, production efficiency factor (PEF) and motility rate were recorded. The data thus obtained were analyzed using Completely Randomized Design (CRD) and means were compared through Duncan Multiple Range test using SAS 9.1. On the basis of different rearing systems, birds reared Cage/ Floor showed better performance with significantly ( $P<0.05$ ) improved FCR, less feed intake, higher body weight and overall lowered mortality rate as compared to other treatments.

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### **CARCASS CHARACTERISTICS, IMMUNE RESPONSE AND ORGAN WEIGHT INFLUENCED BY DIFFERENT HOUSE ZONES IN SEXED BROILERS**

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The present study was undertaken to examine carcass characteristics and organ weight in 720 sexed broilers (360 of each sex) maintained over three different house zones (Pad zone, Central zone and Fan zone) having 4 feeding regimes from 0-10, 11-20, 21-34, 35-42 days with CP levels of 21, 20, 18 and 17 percent, respectively. At the termination of the trial, all the treatment groups received the same weighed average of 19 % crude protein and 2800 Kcal/kg metabolizable energy (ME). At 6<sup>th</sup> week of age, 6 birds/ treatment group were slaughtered according to Halal Muslim method and internal organ weights and carcass characteristics were examined. Statistical analysis of data through CRD under factorial arrangement and comparison of mean using Duncan's Multiple Range test with the help of SAS 9.1 revealed significant effect ( $P<0.05$ ) of different house zones on live, dressed weight, breast meat, thigh meat yield, proventriculus, liver, bursa, lungs, spleen, gall bladder, intestinal weight, shank length, blood glucose and blood cholesterol level of the birds. However, weight after bleeding, blood (% of live weight), gizzard weight (filled, empty), digesta, kidney, pancreas, and heart weight, abdominal fat and intestinal length remained non-significant. The anti-body titer of ELISA for IBD and HI for ND were significantly affected by three house zones while sex had no significant ( $P>0.05$ ) effect on blood urea level, dressing percentage, abdominal fat yield and all visceral organs. The overall results suggested that the broilers reared near pad zone had better dressing percentage and carcass yield.

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### **SUPPLEMENTATION OF SELENIUM SOURCES ON PRODUCTION PERFORMANCE OF INDIGENOUS ASEEL VARIETIES**

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Present study was conducted to evaluate the effect of different Selenium (Se) sources on production performance of four varieties of Aseel viz. Mushki, Lakha, Mianwali and Peshawari at Indigenous Chicken Genetic Resource Centre, UVAS Lahore for the duration of 8 weeks. For this, a total of eighty four adult Aseel females were fed 0.06 % organic and 0.02 % inorganic Se respectively compared with control group and its effect on production performance were recorded. The data were analyzed according to completely randomized design under factorial arrangement with the comparison of mean through Duncan's Multiple Range test with the help of SAS 9.1 revealed significantly higher production % and egg mass of birds fed organic Se whereas among different varieties, Mianwali Aseel showed significantly higher feed intake and egg weight. Other parameters showed non-significant differences among varieties and Se sources. It is concluded that organic Se had overall better production performance in Mianwali Aseel.

### **COMPARATIVE EVALUATION OF POST-PEAK EGG QUALITY PARAMETERS OF FOUR VARIETIES OF ASEELS IN THREE DIFFERENT PRODUCTION CYCLES AFTER INDUCED MOLTING**

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The present study was conducted on Egg quality parameters of indigenous chickens to evaluate the post-peak performance at three different production cycles after induced molt with the objectives to pave the way for improvement in these varieties into sustainable income in favour of the small-scale urban, semi-urban and rural households in the study areas. A total of 96 birds from 4 varieties of Aseel (Mushki, Lakha, Mianwali and Peshawari) X 3 production cycles (3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>) X 8 replicates (individual bird/replicate) were evaluated during Post-peak phase after induced molting for egg quality

parameters. Following characteristics were evaluated: egg weight (g); egg shape index (%); egg specific gravity (g/cm<sup>3</sup>); shell thickness (mm); yolk, white and shell weight (g) and their percentage in the egg mass; yolk index (%) and the number of Haugh units. The data thus collected were analyzed by ANOVA for a completely randomized design, using GLM procedure of SAS 9.1. Results of the present study revealed that significant difference were found among the varieties with highest values of higher albumen ( $P < 0.01$ ) and shell ( $P < 0.05$ ) percentages in the Peshawari were observed, whereas the Lakha had a higher yolk index ( $P < 0.01$ ), higher yolk percentage ( $P < 0.01$ ), and higher yolk-to-albumen ratio ( $P < 0.001$ ). Relative albumen weight was significantly decreased ( $P < 0.05$ ) in 5<sup>th</sup> production cycle, while no significant result were found among 3<sup>rd</sup> and 4<sup>th</sup> cycle. Smaller proportion of yolk and a greater proportion of albumen were found in third production cycle as compared to 4<sup>th</sup> and 5<sup>th</sup>. Production cycle had significant ( $p < 0.01$ ) effect on all the parameters studied in Aseel chicken. Haugh unit score was better in 3<sup>rd</sup> production cycle. The investigations proved that as the production cycle of hens progressed, the weight of their eggs increased. No negative relationship between the production cycle of hens and shell quality (weight, thickness) was observed.

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**SELECTION FOR HIGHER 4-WEEK BODY WEIGHT IN FOUR CLOSE-BRED  
PARENT STOCKS AT THREE AGES OF JAPANESE QUAIL IN 4<sup>TH</sup>  
GENERATION. 1. EFFECT ON PROGENY GROWTH**

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Present study was planned to evaluate the effect of selection for higher 4-week body weight in the parents of 4 close-bred stocks (CBS) at 3 ages of Japanese quail in 4<sup>th</sup> generation. 540 Japanese quails already selected for higher body weight at three different ages (10, 12 and 14 weeks) from four CBS (Major, Kaleem, Saadat and Zahid) were subjected to 3 selection methods (*i.e.* pedigree, mass selection and random-bred control). The effect of selection method was measured on the subsequent progeny growth. Significant effect of selection on feed intake (g), body weight gain (g), and FCR was observed in pedigreed birds followed by mass selected and random-bred control. Age was also found to have significant effect on body weight gain (g) and FCR. It was concluded that pedigree based selected bird's progeny showed significantly better growth performance.

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### **EFFECT OF VARYING LEVELS OF CONCENTRATE RATION ON THE PERFORMANCE OF NILI-RABI BUFFALO HEIFER CALVES**

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The current study was conducted to set the appropriate concentrate level for Nili-Rabi buffalo heifers. Twenty seven buffalo heifers were randomly divided into three different groups A, B and C having nine animals in each group. All the heifers were given free access to chopped green fodder and fresh water. In addition, heifers of group A, B and C were given concentrate at the rate of 0.5%, 1% and 1.5% of their body weight. The average daily dry matter intake was 2.69, 3.06 and 3.83 kg with average daily gain of 456.09, 398.56 and 515.87 gm in group A, B and C, respectively. The feed conversion ratio of heifers of these groups was 5.89, 7.74 and 7.52, respectively. There was non-significant ( $P>0.05$ ) difference in the body measurements (height at wither, body length and heart girth), final body condition & scoring and blood serum (glucose, total protein and cholesterol) of heifers of all the three groups. The results of current study shows that there is non-significant ( $P>0.05$ ) difference in the growth rate of Nili-Rabi heifers at varying levels of concentrate so, it is cost effective to raise 6-8 month calves by offering concentrate at the rate of 0.5% body weight along with free access of green fodder.

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### **FROTH STABILITY OF WHIPPED CREAM**

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Whipped cream is enormously used as a dressing agent on a wide variety of bakery products, salads and desserts etc. Universally, it is prepared from pasteurized or UHT treated cream with a higher fat content (35-40%). Traditionally homogenized cream is used as a substrate for the manufacturing of whipped cream, the negative impact of homogenization on froth stability has been well understood however, little is known

about the impact of low pressure homogenization on froth stability of whipped cream during the storage period. The size of fat globules, surface protein content and additives have great effect on overrun and foam stability of whipped cream along with other factors such as whipping conditions, storage temperature, syneresis etc. This review describes the convention of composite techniques in whipping with relation to the effective behavior of homogenization pressure, fat globules size and also the effect of different stabilizers and processing condition on foam stability of whipped cream from present and future perspectives.

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#### **FACTORS CONTRIBUTING TO KID MORTALITY IN GOAT KEPT UNDER FARM CONDITIONS**

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Data of 3917 goat kids was collected from Livestock Experiment Station, Rakh Kherewala, District Layyah for the period from 1998 to 2007. The records were used to evaluate some environmental factors affecting kid mortality rate from birth to weaning of Beetal, Teddy, Nachi, Pak-Angora and Dara Din Panah goat breeds. The overall kid mortality in various breeds as observed in this study was 5.7, 8.3, 10.7, 4.8 and 12.4 per cent for Beetal, Teddy, Nachi, Pak-Angora and Dara Din Panah, respectively. The overall sex wise kid mortality among different breeds averaged 4.2 and 2.9 per cent in male and female sucklers, respectively. Age wise kid mortality up to the weaning age (120 days) was studied. The frequency of kid mortality 0-1, 1-2, 2-3 and 3-4 months of age averaged 35.54, 24.52, 20.94 and 19.00 per cent, respectively. The kid mortality averaged 0, 1.5, 3.8 and 1.8 per cent among kids having birth weights from 0-1.0, 1.01-1.50, 1.51-3.0 and above 3 kg, respectively. Among types of birth (single, twin and triplet), the overall mortality rate averaged 4.5, 2.3 and 0.2 per cent, respectively. Of the total kids born (3917), 12.7 per cent died in winter, 26.2 per cent in spring, 37.5 per cent in summer (dry hot), 18.2 per cent in summer (humid hot) and 5.5 per cent in autumn. It is concluded that effective management in respect of feeding, housing and disease prevention before kidding and care of kids from birth to 4 months of age along with controlled breeding program may be helpful in reducing the mortality rate among these breeds at Rakh Kherewala, District Layyah.

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**ESTIMATION OF ORGANIC AND INORGANIC COMPONENTS OF *CITRUS SINENSIS* LINN (MUSAMBI) AND ITS EFFECTS ON EARLY GROWTH OF BROILER CHICKS**

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The utilization of the waste of *Citrus sinensis* (Musambi) was carried out in the form of poultry feed additive. The work was done in order to analyze the percentage of organic and inorganic components of *Citrus sinensis*. The dried samples of citrus waste were prepared from various dehydration techniques that included sun drying, oven drying and calcium oxide drying. The analysis was carried out for the various contents (*i.e.*, moisture, mineral, fat, protein and fiber content). The same samples were then used in poultry feed so as to observe its effects on early growth of broiler chicks. In the component analysis no significant difference was observed in the percentages of moisture, fat, protein and fiber but in the mineral content of the samples treated with calcium oxide that was observed to be higher. In case of broiler chicks the growth effects were observed in three groups' *i.e.*; A (control), B (5% citrus content), and C (10% citrus content). A slight decrease in the weight of the chicks was observed with an increase in the concentration of citrus content.

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**RELATIONSHIP BETWEEN EGG WEIGHT AND GROWTH TRAITS IN *PHASINUS COLCHICUS* FED WITH POULTRY FEED IN CAPTIVITY**

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The effect of egg weight on growth traits of ring necked pheasants from day-old chick to 3-month stage was observed in captivity. The breeder pheasants were fed with poultry feed and a total of 129 eggs of ring necked pheasants were collected from Captive Breeding Facilities for Pheasants, Ravi Campus, University of Veterinary and Animal Sciences, Lahore. Each egg was weighed and its length and breadth were taken. Pheasant eggs were divided into three weight groups and were classified as light (20.0 to 26.0g),

medium (27.0 to 32.0g) and heavy (33.0 to 39.0g) weight category and ten eggs were selected for each category at random. The eggs were incubated in Victoria incubators under standard conditions of incubation and Out of total 129 eggs, 61 hatched successfully. Ten chicks from each of the three egg weight categories were selected at random and chick weight, wing length and wingspan of each of these chicks were taken at the time of hatching and thereafter the increase in weight, wing length and wingspan was noted on weekly basis. The chicks were fed with poultry feed throughout the study period. The effect of egg weight on chick weight, live weight gain, wing length and wingspan was found significant ( $P < 0.05$ ). Average chick weight at hatching in heavy, medium and light weight egg groups was 17.7g, 15.0g and 12.7g, respectively and the average live weight at three months stage was 612.4g, 529.1g and 473.6g, respectively. Our studies reveal that egg weight has pronounced effect on growth traits of ring necked pheasants.

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**EFFECT OF SUPPLEMENTING DIFFERENT LEVELS OF MANAN OLIGOSACCHARIDES (MOS) ON BODY MEASUREMENTS AND LYMPHOID ORGANS OF JAPANESE QUAIL**

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Present study was conducted to evaluate the effect of Manan Oligosaccharides on body measurements and lymphoid organs of Japanese quail at Avian Research and Training (ART) Centre, University of Veterinary and Animal Sciences, Lahore Pakistan. For this purpose, a total of 480 quail chicks were procured from ART Hatchery comprising four experimental unit (0.25% MOS, 0.5% MOS, 1% MOS and Control group) having 6 replicate of 20 birds each. Birds were reared in octagonal broiler quail house at standard managemental conditions. At 4<sup>th</sup> week of age, three birds from each replicate were slaughtered and their body measurements and lymphoid organ weight were recorded. Statistical analysis of data in Completely Randomized Design (CRD) through one-way ANOVA technique while comparison of mean through Duncan's Multiple Range test revealed significantly higher body weight, feed intake, Thymus, and Bursa weight in birds fed 0.5% MOS level. Shank and drum stick length and wing spread were higher in birds fed 1% MOS levels. It is concluded that birds fed 0.5 and 1% MOS level had significant effect on body measurements and lymphoid organs weight.

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### **EFFECT OF AGE AND GENETIC VARIATION ON OVERALL PRODUCTIVE AND REPRODUCTIVE PERFORMANCE IN JAPANESE QUAIL**

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The experiment was conducted to evaluate the effect of genetic variation (different close-bred stocks) and age groups on productive performance and hatching traits of Japanese quails at Avian Research and Training Centre, UVAS Lahore. The experiment was conducted on three age groups (A (21), B (19) and C (17) weeks) and four Close-Bred Stocks (Major, Zahid, Kaleem and Sadaat) of Japanese quail. The data were analyzed using SAS 9.1 version for windows using factorial ANOVA. Post hoc analysis was conducted using DMR test. The results of present study showed significant differences among different age groups in egg production (%), egg weight (g), egg mass per bird (g), settable egg (%), FCR per dozen, Feed intake (2<sup>nd</sup> week), hatchability (%), dead in shell (%), dead in germ (%), Clear egg (%), fertility and Hatch of fertile (%). The CBS× age interaction also showed significant variation in egg mass per bird, and FCR per dozen of breeder quails. Regarding hatching performance, dead in shell (%), clear egg (%) and fertility (%) also showed significant variation as a response interaction between different age groups and close-bred stocks.

### **ESTIMATION OF GENETIC PARAMETERS IN RESPONSE TO SELECTION FOR HIGHER THREE WEEK BODY WEIGHT IN JAPANESE QUAIL**

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The present experiment was conducted at Avian Research and Training Centre, University of Veterinary and Animal Sciences, Lahore in order to estimate some genetic parameters for body weight, egg production performance, egg quality and hatching traits in Japanese quail selected for three generations for higher body weight. The univariate

analysis of different traits included generation, sex and genetic group as fixed effects. WOMBAT computer software for Restricted Maximum Likelihood (REML) procedure was used to analyze data from a fully pedigreed population of Japanese quail. Records of 480 animals with 81 sires and 200 dams were used for this purpose. The heritability estimates of body weight at the age of 3<sup>rd</sup>, 4<sup>th</sup> and 6<sup>th</sup> weeks were 0.0126°, 0.0315° and 0.20° respectively. For egg production traits including age at 1<sup>st</sup> egg, total eggs produced and average feed per egg, these were 0.20°, 0.20° and 0.0047° respectively. Whereas for fertility and clear eggs %, heritability estimate was 0.0315°, while it was 0.000° for hatchability %, showing the impact of environment more pronounced than genetics on hatching traits. Breeding values for growth performance in selected birds at the age of three weeks ranged between, -0.1845 to 0.4102, -0.2309 to 0.471 and -0.0908 to 0.5373 in generation 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> respectively. For random-bred control birds the values ranged between -0.845 to 0.6384, -1.2542 to 0.6713 and -1.3781 to 0.7623 for 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> generation respectively. Estimation of genetic trend with the help of breeding values showed positive response to selection as the generations progressed.

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**FORMULATION OF FEED FROM HYDROLYZED CHROME SHAVINGS  
REPLACING COMPLETE VEGETABLE PROTEIN SOURCE FOR QUAILS  
(COTURNIX COTURNIX JAPONICA)**

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The present study was carried out to investigate different chrome shaving levels (tannery waste) on the growth performance of quail chicks. The tannery waste (chrome shavings) was replaced by the vegetable proteins and two different levels were prepared i.e 7.5% , 10% and then added in the feed of quails. 500 quail chicks were purchased from a local hatchery and randomly divided into three groups, one is control(0%) and designated as A, whereas second is experimental group(7.5%) and designated as B and the third is also experimental group(10%) and designated as C. The growth performance of the chicks was evaluated upto the 9<sup>th</sup> week after hatching. After slaughtering the blood was collected for further analysis of different haematological parameters like Hb, TEC, TLC and PCV. There is a significant increase in the levels of Hb (hemoglobin) , TEC (Total Erythrocyte Count), TLC (Total Leucocyte Count) and PCV (Packed Cell Volume) in the 7<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> weeks of both the 7.5% and 10% of chrome shavings as compared to control 0%. The changes were time and dose dependant.

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**SOME ASPECTS OF REPRODUCTIVE POTENTIALS OF A CAPTIVITY  
MAINTAINED STOCK OF ALECTORIS CHUKAR: HATCHABILITY AND  
SURVIVAL RATE**

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<sup>2</sup>*Bioresource Research Center (BRC), 34-Bazar Road Islamabad*

Eggs (n 348) obtained from 48 - 60 weeks old chukar partridge (*Allectoris chukar*) were analyzed for the morphological features of eggs viz egg shell color (EC), egg length (EL), egg breadth (EB), egg shape index (ESI), shell thickness (ST), membrane thickness (MT) and their relation to hatchability, fertility and survival rates. Based on the morphological features eggs were categorized infertile, hatched and unhatched eggs. Egg shell coloration and pigmentation significantly ( $p < 0.05$ ), produced an obvious effect on the fertility and hatchability of eggs. The LCSS (Light Creamy with Small Spots) eggs showed higher fertility and hatchability rate. EL, EB and ESI shows no significant effect on the % hatchability in chukar eggs. ST has showed a significant difference between the hatched, unhatched and infertile eggs while MT shown no significant difference between the infertile and hatched eggs. While MT varied significantly between hatched and unhatched eggs. Fertility and hatchability of *Alectoris chukar* are also affected by ST and MT. Temperature is an important physical factor that determines survival rates in *Alectoris chukar*. The egg production rate was higher in mid-March. Two peaks in mortality occurred first in the month of April and second in months of June and July. As an outcome of this study, it is clear that chukar partridge can be raised under intensive captive conditions when some management techniques are applied.

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**ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF HEAVY  
METAL RESISTANT BACTERIA FROM INDUSTRIAL EFFLUENTS**

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Sixteen heavy metals resistant bacterial strains isolated from industrial waste waters of district Lahore and suburbs were found to be tolerant to multiple heavy metals like Cr, Ni, Pb, As, Cu and Hg on agar media. Optimum growth temperature and pH for most of these strains were 30 °C and 6.5. These bacterial strains were found to reduce 60-88% of different metals from culture media after 72 hours while performing 20.2-40.1% metal accumulation for different metals after same hours of inoculation. Scanning electron microscopy revealed signs of metals adsorption at bacterial cell surfaces. SDS-PAGE also indicated enhanced expression of low molecular weight proteins (metallothionein) after treatment of bacterial cells with heavy metals in solution. Generally higher resistance was found against Pb, Ni and As in the identified bacterial

strains like *Klebsiella* sp., *Staplococcus* sp., *Pseudomonas aeruginosa* and *Bacillus licheniformis*. All of these strains were found to have plasmid while most of these had chromosome based metal resistance except *Pseudomonas aeruginosa* and *E.coli* that were found to have plasmid based resistance for chromium. Copper resistance gene *CueR* was detected in copper resistant bacterial strain *Klebsiella* Sp. which was also found to be positive for mercury resistance gene (*merA*) and arsenic resistant gene (*arsC*). *merA* was also found to be present in *Staplococcus* sp.. In addition to *Klebsiella* sp. *arsC* gene was also detected in *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. Isolation of these genes and their further characterization can be helpful in designing of an efficient heavy metals detoxification strategy. In the light of above observations present study can be helpful for efficient exploitation of bacterial strains for the purpose of biological waste water treatment.

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#### EVALUATION OF EFFICACY OF KILLED AND COMMERCIALY AVAILABLE LIVE NEWCASTLE DISEASE VACCINE IN BROILER CHICKENS IN PAKISTAN

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The study evaluated the efficacy of Newcastle Disease killed and commercially available live vaccine. Moreover, study also highlighted the inappropriate use of Newcastle Disease live vaccine as a booster dose providing the space to virus for its growth and multiplication in semi controlled broiler farms creating threat to whole poultry industry. Serum antibodies level was assessed by Haemagglutination Inhibition method and efficacy of Newcastle Disease killed and live vaccine was also determined using challenge test. Group "A" immunized with killed vaccine using homologous strain provided 90% to 100% protection. However, group "B" immunized with Newcastle Disease live vaccine (Heterologous strain) provided protection not more than 60% when challenged with velogenic viscerotropic form of Newcastle Disease Virus isolated from the recent outbreak. The results of the present work concluded that oil emulsified killed vaccine provided good protection using indigenous isolate against Newcastle Disease. Regression analysis showed highly significant ( $P < 0.001$ ) correlation between antibodies titre against Newcastle Disease versus age of the flock in group immunized with killed vaccine and significant ( $P < 0.01$ ) correlation in that group immunized with live vaccine.

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## SECTION – I I

### PESTS AND PEST CONTROL

#### EVALUATION OF WHEAT LINES FOR RESISTANCE AGAINST LEAF APHID (*ROPHALOSIPHUM MAIDAS*)

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Evaluation of wheat lines for resistance against aphid species was done in one hundred and twenty four wheat lines against Corn Leaf Aphid (*Rhopalosiphum maidas*) at National Agricultural Research Centre, Islamabad. Three tests viz. Seedling Bulk Tests, Antibiosis and Antixenosis were used. Data in Seedling Bulk Test were recorded on the basis of visual damage rating scale 0-9. Results showed that six lines 11129, 11123, 11042, 11039, 11037 and 11034 were resistant with damage rating 1-3. Ninety one lines were moderately resistant with damage rating of 4-6 and remaining twenty three lines were susceptible with damage rating scale 7-9. Four resistant, 3 moderately resistant and 3 susceptible lines were selected for other two tests. In case of antixenosis (non preference) studies, out of 10 selected lines, 3 lines, 11034, 11046 and 1106 were least preferred by *R. maidis*. Most preferred lines, not suitable for our breeding programs against this pest were 11129 and 11049 and 5 lines were moderately preferred. In antibiosis, data regarding fecundity (number of nymphs laid) were recorded on the same 10 selected lines. Results of this study showed that 5 lines 11034, 11037, 11039, 11062 and 11079 were least fecund, 4 lines were moderately fecund and one line 11029 was highly fecund.

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#### BIO-INTENSIVE BASED INTEGRATED PEST MANAGEMENT AGAINST INSECT PESTS OF TOMATO UNDER NATURAL FIELD CONDITIONS

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Four tomato cultivars viz., nagina, riogrande, pakit and tomato-7 were planted in a field trial to study bio-intensive integrated pest management of aphids and fruit borer

pests during the year 2010. Crop was raised under standard agronomic practices throughout the growing period. Pests of crop were managed by the releases of bio-control agents, parasitoids (*Trichogramma chilonis*), predator (*Chrysoperla carnea*) and application of insecticide sprays in comparison to untreated control. Aphid population was reduced from 22.3 (untreated control) to 1.5/leaf with six weekly releases of *C. carnea* and two insecticide sprays. Infestation of tomato fruit borer was minimized from 1.5 larvae/plant (untreated control) to 0.25 larvae/plant (treated) with two releases of parasitoid *T. chilonis*.

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### **SUSCEPTIBILITY OF *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) TO DELTAMETHRIN IN DISTRICT SARGODHA**

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Present study was conducted to determine the resistance status of *Culex quinquefasciatus* against deltamethrin (0.5%). For the study blood fed female mosquitoes were sampled from the old Medical College Building, University of Sargodha and subjected to residual bioassays according to standard protocol of WHO. Activities of estrases, monooxygenases and glutathione-S-transferases (GSTs) were also determined. For the biochemical estimation of estrases, (GSTs) and mono-oxegenases methods described by Baker *et al.* (1998), Habig *et al.* (1974) and Vulule *et al.* (1999) were followed. The mortality rate was 100% after 24 hours of deltamethrin (0.5%) exposure. Results of residual bioassays revealed that mosquitoes were susceptible to recommended dose of deltamethrin (0.5%). Calculated LT<sub>50</sub> was 6.38 hours. Non-significant difference was observed in the activities of enzymes in control and treated groups of *C. quinquefasciatus*. It is concluded that mosquito population in the study area is not resistant to the recommended dose of deltamethrin

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### **PREVALANCE OF INSECT PESTS OF SUGARCANE IN DISTRICT SWABI**

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<sup>2</sup>*National Agriculture Research Centre (NARC), Islamabad*

The present study was conducted on prevalence of insect pests of sugarcane in District Swabi, KP, Pakistan. Sugarcane (*Saccharum officinarum* L.), is a high value cash crop of Pakistan. It plays an important role in the up lift of socioeconomic conditions of the growers. Collection was carried out during September 2013-February 2014. This was the first survey of insect pests of sugarcane of District Swabi. The specimens were collected with the help of scoop net, sweep net, wet finger, fine-hair brush, forceps and

directly by hands. The collected samples identified with the help of taxonomic keys and preserved specimens in National Insect Museum at NARC Islamabad. Monitoring of pests of sugarcane crop in the mentioned area during the study period showed the occurrence of top borers, stem borers, root borers, sucking insects, flies (white fly), leafhopper, mealy bugs, black bugs etc. This study will help to understand and control the pests of sugarcane in Swabi.

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**EFFECT OF SOME DIPTERAN HOSTS ON THE BIOLOGY OF *DIRHINUS GIFFARDII* UNDER LAB CONDITIONS**

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Results of studies on the host suitability for the parasitoids are of paramount importance for the economical mass rearing and implementing Integrated Pest Management (IPM). The *Dirhinus giffardii*, a pupal parasitoid attacking a number of Dipteran fly species. We investigated the effect of different Dipteran hosts on the biological parameters of *D. giffardii* under laboratory conditions. The pupae of *Musca domestica*, *Bactrocera cucurbitae*, *Bactrocera zonata* were offered to *D. giffardii*. Results revealed that significantly ( $p < 0.05$ ) higher parasitism rate with maximum longevity, fertility and fecundity were observed when the pupae of *M. domestica* were offered to pupal parasitoid. In contrast to this, significantly lower parasitization, short longevity, lower fertility or fecundity were recorded when pupae of *B. cucurbitae* and *B. zonata* were provided to *D. giffardii*.

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**RESPONSE OF EARLY, MID AND LATE MATURING SUGARCANE VARIETIES ON THE POPULATION DYNAMICS OF AN EGG AND A LARVAL PARASITOID ON STEM BORER *CHILO INFUSCATELLUS***

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In a study conducted at Nuclear Institute of Agriculture Tandojam Sindh, Pakistan, during 2011, eight sugarcane varieties were selected with an objective to assess the varietal effect on the population dynamics of sugarcane stem borer and its parasitoids. Results revealed that pest population was the lowest in the month of February and highest infestation was observed in October in all varieties. The minimum mean borer infestation percentages were observed in both late maturing varieties Disco (B-43/60, 9.62%) and SPF 238 (11.05%) whereas, early maturing and high sucrose content variety SPSG-26

showed the maximum infestation of 23.7%. Parasitism percentages by the egg parasitoid *Trichogramma chilonis* and *Cotesia flavipes* were higher on varieties with higher infestation levels except Disco (B-43/60) with comparatively higher parasitism levels (10.02% For *T. chilonis* and 6.8% for *C. flavipes*) in spite of lower infestation level. This was probably due to the morphological characteristics of plant. None of the varieties showed complete resistance to borer infestation. Varietal resistance can be a major contributing factor in reducing borer infestation.

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**TOXICITY OF *AZADIRACHTA INDICA* (NEEM) AGAINST *TRIBOLIUM CASTANEUM* (RED FLOUR BEETLE) INFESTING RODENT POISON BAITS**

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The anticoagulants are being used for rodent control since last three decades. Rat baits containing anticoagulants become infested by insect pests such as, red-flour beetle, *Tribolium castaneum*. Materials used for bait formation and taste additives to attract rodents may be more attractive for the insect pests. Moreover, stored food commodities may become infested with insects introduced by anticoagulants. Many researchers have stressed on urgent need for addition of invertebrate antifeedants, palatable to rodents in anticoagulants baits. Neem derivatives have antifeedant and insecticidal activities against insect pests. In this regard, a study on the toxic effects of neem (*Azadirachta indica*) seed 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. For testing neem seed powder, wheat flour, broken rice, additive (egg), poison and neem seed powder were mixed in a ratio accordingly. All ingredients were mixed w/w. Neem seed powder was tested in three (2%, 1% and 0.5%) doses, in comparison with control (plain bait). Fifty grams of treated and untreated (control) bait was kept in 450 gm capacity glass jars covered with muslin cloth. After two days, twenty, 10 days old adults of red flour beetle, *Tribolium castaneum* were released in each jar. All the trials were replicated four times, under the same temperature and humidity. Mortalities were recorded after 24, 48 and 72 hours. Motilities were observed at all doses, 100% at 2%, followed by 89% at 1% and 61% at 0.5% in 24 hours; 100% at 1% in 48 hours; 100% at 0.5% in 72 hours. The study may be useful in utilization of neem seed powder for the protection of anticoagulant baits from the infestation of red flour beetle, *Tribolium castaneum* and its use can be a better solution to increase bait shelf life.

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**THE MACROINVERTEBRATE COMMUNITIES ASSOCIATED WITH SOME WEED PLANTS OF SUGARCANE (*SACCHARUM OFFICINARUM*) AND WHEAT (*TRITICUM AESTIVUM*) CROPS OF FAISALABAD DISTRICT (PAKISTAN)**

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Sweep-net sampling was used to collect macroinvertebrates from weeds occurring in the edges and inside the fields of both wheat and sugarcane agro-ecosystems. The main objective of the recent study was to determine comprehensive relationship between macroinvertebrate fauna and weed species associated to wheat and sugarcane agro-ecosystems. A total of 2,468 and 2,963 specimens of macroinvertebrates including arthropods and pulmonates, were recorded both from wheat (62 species) and sugarcane (162 species) associated weeds. *Cynodon dactylon* supported most diverse and abundant assemblage of macroinvertebrates in both wheat and sugarcane. It shared 52.02% of total collected individuals in sugarcane and 33.47% in wheat and Orthoptera, Hemiptera, Diptera, Hymenoptera and Araneae were the most abundant taxa. Edges of both wheat and sugarcane fields were significantly rich and diverse ( $S = 60$ ,  $H' = 3.16$ ), ( $S = 149$ ,  $H' = 4.05$ ) than in the centers ( $S = 38$ ,  $H' = 2.93$ ), ( $S = 79$ ,  $H' = 3.56$ ), respectively. Weeds were found important in sustaining biodiversity and richness of macroinvertebrate species that in turn helped in the sustainability of agro-ecosystem.

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**INHIBITION OF ACETYLCHOLINE ESTERASES OF *TRIBOLIUM CASTANEUM* (LARVAL AND ADULT STAGES) BY COMPOUNDS DERIVED FROM A MEDICINAL PLANT *AZADIRACHTA INDICA***

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*Tribolium castaneum*, a stored grain pest, is responsible for severe damage to stored grain. A number of chemical based sprays are used to control the pest attack. These synthetic compounds are hazardous to friendly insects and human beings. It is required to use natural and degradable bio safe pesticides. The molecular basis of the pest control is dependent on the action of insecticides on the central nervous system enzymes in the insect. Acetylcholinesterase (AChE) is a neurotransmitter that is involved in the

rapid hydrolysis of acetylcholine (mainly present at the neuro junctions and synapse). The study was undertaken to determine the effect of plant derived molecules at larval and adult stage of *T. castaneum*. A traditional medicinal plant *Azadirachta indica* was selected for the study. Two groups of *A.indica* derived compounds 1: Saponins and 2: Azadirachtin, isolated and purified from the plant tissues (leaves and seeds), were investigated. Saponins and azadirachtin were tested for the inhibition of enzyme acetylcholine esterase in the adult and larval stages of beetle, *T. castaneum*. Saponins inhibit the enzyme activity to a greater extent. Insects at larval and adult stages were repelled by saponins and azadirachtin. The results showed that the *A.indica* compounds could be used as biopesticides.

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**EFFECT OF ARTIFICIAL DIETS AND FLORAL NECTARS ON THE BIOLOGY OF *TRICHOGRAMMA CHILONIS* (ISHII) (HYMENOPTERA: TRICHOGRAMMATIDAE) UNDER LABORATORY CONDITION**

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*Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) is the most widely used biological control agent against Lepidopteran pests. Adult wasps prefer sugar-based substances as food, especially plant nectar. Therefore, this study was carried out to evaluate the best artificial diet and floral nectars for the mass rearing of *T. chilonis*. The experiment was conducted in the biological control laboratory of Plant Protection Division at Nuclear Institute of Agriculture (NIA), Tandojam by testing the different floral nectars (Marigold and rose flower) and artificial diets (honey solution, sugar, glucose, protein hydrolyzate and egg yolk) on the parasitism, adult emergence and sex ratio of emerged parasitoids. The results revealed that *T. chilonis* females fed on rose nectar parasitized host eggs significantly higher in number ( $69.2 \pm 2.76$ ) with maximum adult emergence % ( $79.4 \pm 1.46$ ) as compared to female fed on marigold ( $55.4 \pm 2.42$ ) ( $68.8 \pm 2.78$ ). Whereas the *T. chilonis* females that fed on honey parasitized ( $86.6 \pm 2.18$  eggs) ( $93.2 \pm 1.65$ ) followed by sugar ( $80.6 \pm 2.54$ ) ( $86.6 \pm 2.42$ ), glucose ( $79.2 \pm 1.65$ ) ( $83.40 \pm 3.58$ ), protein hydrolyzate ( $62.2 \pm 1.65$ ) ( $76.2 \pm 3.20$ ) and egg yolk ( $54.60 \pm 2.01$ ) ( $69.6 \pm 4.22$ ) parasitized significantly more hosts than females that fed on other floral nectars and artificial diets. The females fed on water were shown to have the least parasitization ( $29.2 \pm 1.65$ ) and adult emergence ( $66.2 \pm 2.63$ ). Different artificial diets and floral nectars have no effect on the sex ratio of *T.chilonis*. It can be concluded from the results that honey can increase the performance of the wasp parasitoids. Although qualitative floral nectar may be a great importance to parasitoid for conservation but the artificial diets proved to be suitable foods for sustaining the development and reproduction of *T. chilonis*.

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**APHID INFESTATION TO DELAYED SOWN CANOLA, *BRASSICA NAPUS* L.  
REDUCES CHLOROPHYLL CONTENTS BUT NOT PHOTOSYNTHETIC  
ACTIVITY**

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Canola, *Brassica napus* L. crop is attacked by a number of insect pests among which cabbage aphid, *Brevicoryne brassicae* L. and turnip aphid, *Lipaphis erysimi* (Kalt.) are responsible for causing heavy yield losses, especially in Southern Punjab of Pakistan. We determined seasonal incidence of aphids and their effects on different photosynthesis parameters including photosynthetic rate, water use efficiency transpiration rate, total internal CO<sub>2</sub> and chlorophyll contents on five cultivated varieties in Multan during 2011. Five canola varieties were sown on in the last week of November 2010. Treatments (varieties) were arranged in a randomized block design with three replications. Each treatment was divided in to two parts, imidacloprid was applied on one part on weekly basis from initiation of aphids for keeping the crop aphid free while other part was left untreated to observe aphid infestation under natural conditions. Aphid population was noted at weekly intervals starting from appearance of aphid on the crop until the maturity. Peak aphid population was observed on the 12th March, 2011. Numbers of aphids were significantly different on two sampling dates out of five in the tested varieties. Aphid infestation reduced chlorophyll contents but did not result in significant reduction in photosynthetic rate, water use efficiency transpiration rate and total internal CO<sub>2</sub>. No differences were observed across the varieties for all photosynthetic parameters in aphid infested as well as aphid free treatments; expect that of chlorophyll contents were significantly higher in aphid protected plants of Parola and Punjab vanities.

**OBSERVATION ON THE APHIDS (HEMIPTERA:APHIDOIDEA) FROM  
KARACHI AND ITS ADJOINING AREAS**

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Aphids are commonly known as the “Plant lice” are sap-sucking creatures. They are the most destructive pest of cultivated crops. They cause damage to the plants and are enemies of the farmers and gardeners throughout the world including Pakistan. At the present it was noted that these creatures are mostly found in the temperate areas of

Karachi and its adjoining areas. Due to their migratory habitat they are able to migrate great distances riding via winds.

### SEASONAL MONITORING OF FRUIT FLY, *BACTROCERA ZONATA* AND ITS PARASITOIDS ON GUAVA FRUIT

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Seasonal monitoring of fruit fly, *Bactrocera zonata* and *B. dorsalis* was studied on infested guava fruits for recording the larval parasitoid under laboratory conditions. The *B. zonata* and *B. dorsalis* pupae were collected between periods from 01.11.2012 to 15.03.2013 at fortnightly interval. The data showed that *B. zonata* adult emergence was  $65.67 \pm 1.69$  percent; while emergence percentage of *B. dorsalis* adult was recorded  $5.54 \pm 2.35$ . It was also found that female ratio was recorded dominates over the male *B. dorsalis*. Similarly, males' adult parasitoids emerged in a fortnight on average ( $3.00 \pm 0.26$ ). Beside this, there was significant difference was noted in the adult emergence in larval parasitoid i-e  $16.26 \pm 1.16$  percent and female ratio was calculated remarkably higher ( $9.16 \pm 0.84$ ) than fruit fly trap catches between weeks ( $P < 0.05$ ). Furthermore, there was positive and significant correlation was found between fruit fly infestation and relative humidity i-e ( $r = 0.7244^{**}$ ), and temperature ( $r = 0.5299^{**}$ ) over all these findings suggested that high relative humidity and temperature was responsible for high infestation of fruit fly.

### FEEDING BEHAVIOUR OF LEAF FOLDER (*CNAPHLOCROSIS MEDINALIS* GN.) AND RICE GERM PLASM PREFERENCE

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Rice yield losses in Pakistan goes up to 30% in major rice growing tracts and causes 15- 25% yield losses in the country in basmati rice alone. Germ plasm generally resists the leaf folder attack owing to their inherent capacity (antibiosis mechanism). In addition to this, narrow leaved varieties are believed to be more resistant to leaf folder



attack as compared to broad leaved varieties. But contrary to this, keeping in view the feeding behavior of leaf folder larvae, it is found that the broad leaved varieties may escape from the ravages of this notorious pest as edges of broader leaves goes out of the reach of the larvae during leaf folding process. During folding of leaf, larvae sits in the center of leaf and anchor on the leaf with half of its body while moves other half from one edge of the leaf to other for fastening to form a longitudinal fold. So broader leaved rice varieties too may be proved as a promising tool of escaping of leaf folder attack and consequently yield loss.

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**CONTRIBUTION OF BLOWFLIES (DIPTERA: CALLIPHORIDAE)  
POLLINATION TOWARDS YIELD ENHANCEMENT IN MANGO  
(MANGIFERA INDICA L.)**

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The blowflies (Diptera: Calliphoridae) were originated better to use and more effective than open pollination in mango orchard blossom pollination. In the present study, the impact of pollination on mango yield by blow fly was studied in the field at Faculty of Agricultural Science and Technology, Bahauddin Zakariya University Multan. The experiment was laid under Randomized Block Design with three treatments *i.e.* a) open pollinated trees, b) trees covered by mosquito net devoid of insects and c) tree covered by mosquito net with blow flies for pollination. All the treatments were replicated thrice. The experiment was started with the emergence of blossom on the mango trees. Trees were fully covered with mosquito net in treatments b and c. Populations of blow flies were ensured during the mango blossom period for pollination. Comparison among the treatments was made by fruit setting, size and weight of fruits. Shapes of blossoms were also categorized and data of no. of flowers on different shapes of blossoms were recorded. Maximum number of flowers were recorded on irregular type of inflorescence *i.e.*  $434.80 \pm 30.14$  flowers/inflorescence as compared to pyramid ( $400.90 \pm 22.4$  flowers/inflorescence) and conical ( $327.97 \pm 14.97$  flowers/ inflorescence). Highest fruit setting *i.e.* 67% was observed in open pollinated trees (pollinated by *Apis dorsata*, *Apis florea*, *Episyrphus balteatus*, *E. scutellaris* and *Lucilia* spp.) followed by 27% more fruit in tree pollinated by blow flies compared to control. There was 37% larger size of fruit in open pollinated mango trees and 15% larger fruit size recorded by blow flies pollination in captivity, compared to control. Maximum premature weight of mango fruit ( $201.19 \pm 1.76$ g) was observed in open pollinated tree. It is concluded that blow flies can be used as an effective mango pollinators along with other bees. With the help of blow flies pollination, yield quality and quantity of mango fruit trees could be enhanced.

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### **PREDATOR-PREY INTERACTIONS OF SYRPHIDS AND APHIDS IN CROPFIELDS OF FAISALABAD, PAKISTAN**

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Predator -prey interaction is one of the key point involve in natural process performed for the stability of ecosystem. In ecological communities, interaction of predator with their prey and their strength varies massively among different species. In present study relatively abundant seven species of predators (syrphids) *Episyrphus balteatus*, *Melanostoma scalare*, *Syrphustorvus*, *Meliscaeva auricollis*, *Sphaerophoria scripta*, *Eupeodes corollae* and *Ischiodon scutellaris* and six prey species of Aphids; *Rhopalosiphum maidis*, *Rhopalosiphum padi*, *Schizaphis graminum*, *Brevicoryne brassicae*, *Myzus persicae* and *Lipaphis erysimi* were selected to determine their predatory interaction among species of these two groups. Linear regression was use to record their strength of association. Maximum association of *M. scalare* with *S. graminum* was observed with R<sup>2</sup>value (0.90). *E. balteatus* was found more significantly associated with most of aphid species as with *R. padi*, *S. graminum* and *M. persicae* with R<sup>2</sup>values 0.86, 0.82 and 0.80 respectively. More or less interaction with selected aphid species was recorded by *E. corollae*. *I. scutellaris* did not show strong association with aphid species. Predator-prey interactions of some other species showed comparatively less significant regression (r<sup>2</sup>) values. Whereas, these interactions, seemed to fit the graphic presentation of "Lotka-Volterra equation" and proves it as potential predator of these species in cropland. These studies are very helpful to devious the control programs of pest management especially in crop fields.

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### **EFFECT OF DIFFERENT ENVIRONMENTAL FACTORS ON THE POPULATION FLUCTUATIONS OF RICE STEM BORERS AND LEAF FOLDER**

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The insects were found for a specific duration throughout the year, however, dominance of insects was comparatively higher from March to May and from August to October, in case of White Stem Borer and Yellow Stem Borer during both the years, 2009 and 2010; while from mid-September to start of April of next year in case of Pink Stem Borer. On comparing the weather parameters, importantly temperature and humidity, it was evident that the insect trap catches were recorded higher within specific range of temperature that varies from 25 to 33°C in case of White and Yellow Stem

Borers, and shows most significant relationship with total insect catch. Maximum trap catches were observed during April where average temperature was 30°C, considering it optimal temperature for insect catch by light traps. However, in case of Pink Stem Borer, insect trap was found to occur below 30°C during all winter season. Correlation analysis of temperature with insect catch clearly accentuates the strong relationship of activity of these insects with environmental temperature. At the same time, it was also discovered that this relationship is within specific temperature range, *i.e.* between upper and lower threshold of activity, below and above of this range, temperature conferred no effect on activity of these insects.

**MORPHOLOGICAL CHARACTERS OF SEEDS ISOLATED FROM INDIAN FLYING FOX (*PTEROPUS GIGANTEUS*) EJECTA AT JINNAH AND LALA ZAR GARDENS, LAHORE**

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Seed size and weight are two important parameters that indicate productivity and quality of seed and these morphological variations may be associated with genetic diversity. Present study was focused to check out the morphological variations in seeds isolated from ejecta of tropical fruit bat, *Pteropus giganteus* roosting at Jinnah and Lala Zar Garden, Lahore. The seed measurements length (mm) and diameter (mm) and weight (g) showed that the seeds of *Artocarpus heterophyllus* (Jack fruit) were longest with maximum average diameter while the heaviest seeds in the present study were of *Naphelium lappaceum* (rambutan) with an average weight of 3.4g.

**INTERACTION OF SELECTED COCCINELLID SPECIES WITH DIFFERENT APHID SPECIES OCCURRING IN BRASSICA CROP FIELDS**

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Biological diversity within ecosystems, including agro-ecosystems, provides a wide range of biological functions and there are some indications that more diverse agricultural ecosystems may enhance natural control of pests. Family Brassicaceae includes a number of species that have considerable nutritional and economic values. The

present study has met the objective of exploring and evaluating arthropod population interactions and suspected synergies for the crop plants in terms of pest reductions. Data was collected with the help of hand net and by hand picking from selected quadrates of 1m<sup>2</sup>. A total of 9067 specimens, 8946 from the crop plants and 121 from the weed plants associated to crop, belonging to 81 species were sampled from *Brassica*-weed complex, of which 1096 were coccinellids along with other predators, 3963 aphids along with other pest/prey and 1729 were found to be omnivores. Cruciferous crops were highly vulnerable to pests like many aphids and some lepidopteran species. Maximum Coccinellids (predator) peaks were observed during March and April because they develop well at 22-25°C and due to the presence of their preferred prey (aphids). Predators dominated the weed plants while prey-pest populations dominated the crop plants throughout the cropping season. Therefore, the study evidenced the synergetic effects of phyto-morphic diversity helping pests control on Crucifers.

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**USE OF SYNTHETIC SEX PHEROMONES FOR MONITORING OF  
*PECTINOPHORA GOSSYPIELLA* (PINK BOLLWORM) (LEPIDOPTERA:  
NOCTUIDAE)**

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Cotton bollworm *Pectinophora gossypiella* (pink bollworm) is one of the major lepidopteron pests of the world and cause significant infestation throughout. In Pakistan especially in Sindh province the problem of Pink bollworm increases every year, so growers could not get final picking. Synthetic sex pheromones were used for monitoring of adult moth population of *P. gossypiella* in four localities of Sindh, Pakistan namely Saeedabad, Halla, TandoAllahyar and Jamshoro. The effect of the environmental factors (Humidity, Rainfall and Temperature) was also observed. During present study it is observed that adult moths were captured almost in same number in all studies localities with little variation, these results give us strong evidence about the importance and presence of *P. gossypiella* in these areas. Adult moth population recorded from August to November and reached its peak in October to November in Sindh, Pakistan. Present study revealed that sex pheromone is a useful technique for control of *P. gossypiella* because in Sindh, Pakistan, every year the grower use a large amount of the insecticides for control of *P. gossypiella*, but they could not succeed to manage the infestation problem of *P. gossypiella* and this indiscriminately usage of pesticides cause many problems such as health issues for local populations, native wild animals, insect become more resistance and loss of predators.

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### LABORATORY EVALUATION OF THE EFFICACY OF MOUSTICIDE AGAINST *AEDES AEGYPTI* LARVAE

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Present study was carried out to evaluate the efficacy of two formulations of Mousticide; wettable powder (20% each of *Bti* WP, TMOF, and yeast) and rice husk (4% each of *Bti*, TMOF, yeast and rice husk) against laboratory reared 1<sup>st</sup>-3<sup>rd</sup> instars of *Aedes aegypti*. Laboratory bioassays were conducted by using standard procedures of WHO (2005) by taking 20 *Aedes aegypti* larvae in 200ml of dose. Various concentrations of the two formulations of Mousticide were prepared in distilled water. Each concentration was replicated three times and three untreated cups were used as control in order to determine their active range and to find the minimum effective dose. Hundred percent mortality resulted with 1<sup>st</sup>-late 3<sup>rd</sup> instars against 0.1 ppm of Mousticide WP at 48h post exposure. However, 100% pupal reduction was observed against Mousticide WP at 24-48h post exposure in all instars. Mousticide RH had no mortality by 48h post exposure and onward mortality was inversely proportional with increasing concentrations. Moreover maximal pupal formation (93%) was also observed against 200 ppm of Mousticide RH. In conclusion, Mousticide WP formulation was 100 times more toxic as compared to Mousticide RH. It appeared Mousticide RH containing 4% each of *Bti*, TMOF and yeast was not bound properly with rice husk or degraded resulting no effect in controlling *A. aegypti* larvae. However, Mousticide WP was recommended to use in natural conditions to evaluate its efficacy against *A. aegypti* larvae.

### EFFICACY OF INSECTICIDES AGAINST CITRUS PSYLLA (*DIAPHORINA CITRI*) IN FIELD AND LABORATORY CONDITIONS.

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The field experiment was conducted on citrus plants to check the efficacy of insecticides against citrus psylla, at Horticulture Research Institute, Jhang road, Faisalabad while lab studies were carried out at Entomological research Institute, Faisalabad. Four insecticides, polytrin-C, lambdacyhalothrin, bifenthrin and imidacloprid

applied, had almost equal effect on the population reduction of citrus psylla on all citrus plants. Trial was laid out in Randomized Complete Block Design (RCBD) having five treatments with three replications in citrus orchard, after three days of spray showed percentage control as 96.91%, 94.33%, 93.83% and 93.06% of following insecticides Polytrin-C, Imidacloprid, Bifenthrin and lambda cyhalothrin respectively, calculated by MSTAT-C. Psylla adults were exposed to different concentrations (500, 400, 300, 200 and 100 ppm) of imidacloprid and bifenthrin, and two controlled conditions (with leaves and without leaves). Both imidacloprid and bifenthrin insecticides proved the most effective against *Diaphorina citri* with LT<sub>50</sub>s of 4 and 5 hours respectively at a concentration of 500 ppm, calculated by probability test with Minitab-15.

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#### **DIVERSITY AND RELATIVE ABUNDANCE OF GROUND FAUNA IN SUGARCANE AND WHEAT AGROECOSYSTEM OF FAISALABAD**

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Recent study focused on the diversity and relative abundance of ground fauna in sugarcane and wheat agroecosystem of Faisalabad. A total of 13417 specimens, of which 4532 from sugarcane whereas 8885 from wheat were collected. Wheat was more successful having 885 specimens belonging to 38 species. Among predators, *Pheropsophus hispanicus* was maximum with 169 specimens followed by *Clubiona phragmitis* with 112 specimens. *Tullbergia granulate* was highly abundant among different pest species with 3162 individuals followed by *Oniscus asellus*, *Onychiurus hortensis*, *Armadillidum vulgarae* and *Sminthurus viridis* with 2806, 2553, 904 and 715 specimens respectively.

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#### **BENEFITS OF LESSER/NO APPLICATION OF PESTICIDES IN MANGO ORCHARDS AT MULTAN**

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HBRI, NARC, Islamabad

World is facing tremendous problem of pollination deficit due to habitat degradation and extensive and intensive use of chemicals etc. It is necessary to create awareness among different stakeholders of advantages of pollination through solid evidence. In this connection a survey/experiment was carried out in Multan areas for effectiveness of lesser application of pesticides in Mango orchards. It was organized in may that farmers were contacted with the start of agronomic practices. It was thrice

replicated with orchards of lesser/no application of pesticides and more application of pesticides each. Agronomical practices on which expenses were incurred in more application of pesticides included irrigation, fertilizer, farm yard manure, fungicide, insecticide, harvesting labor, pruning after harvest, weeding, wooden crates, transportation, land preparation for inter-cropping and seed of fodder. In orchard with lesser/no application of pesticides all practices were same except insecticide spray and weeding was not done. Details of expenditure on various agronomical practices were gathered by personally visiting these farmers' orchards frequently and obtaining the expenditure and sale/profit data. The balance profit was calculated by subtracting expenditure from profit. This was converted to % profit. Yield of each field was also obtained. Yield per acre of Mango was 8000, 9600, 12000 (av.9866) Kg in lesser/no application of pesticides orchards and 5000, 5714, 6400 (av.5704) Kg in more application of pesticides orchards. Thus a benefit of 4162 Kg/acre (av.) was obtained. Profit recorded in orchards with lesser/no use of pesticides was 68.4, 138.8 and 105.2 % (104.1) and in more use of pesticides ~as 32.4, 33.3 and 37.9 % (34.5). Thus margin of profit was much higher in the lesser/no use of pesticides orchards. This was possible due to conservation of beneficial insects (parasitoids, predators, and pollinators).

**DOES THE STARVATION LEVEL AFFECT THE FEEDING POTENTIAL OF  
PREDATORY LADYBIRD BEETLE *COCCINELLA SEPTEMPUNCTATA*  
(COLEOPTERA; COCCINELLIDAE): LIVE VS FROZEN HOST SPECIES.**

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Ladybird beetles/Ladybugs, both adults and larvae, are well-known primarily as predators of aphids (plant lice); however, they also prey upon many other soft bodied insects and eggs of different borers. Laboratory rearing of predatory ladybirds often need a live host particularly aphids. Studies were conducted to check the suitability of live and frozen rose *Macrosiphum rosae* and mustard aphids *Lipaphis erysimi* to rear seven spotted ladybird beetle *Coccinella septempunctata* when fed normally (un starved beetles) or when the beetles were kept starved for 16 hours. Results showed that *C. septempunctata* in both, starved and unstarved conditions consumed more aphids of both species during the 1<sup>st</sup> hour of feeding. There were no significant differences in numbers of live and frozen mustard aphids consumed by *C. septempunctata* when the beetles were unstarved. However, starved beetles consumed more frozen aphids as compared to live (Mean + SE = 6.24 + 0.37 and 2.43 + 0.40 respectively). Likewise, when offered live and frozen rose aphids, alike feeding preferences were shown by starved beetles, consuming more frozen aphids (6.51 + 0.5 (frozen aphids), 4.86 + 0.49 (live aphids)), while,

unstarved beetles consumed similar numbers of live and frozen aphids (Mean + SE = 6.64 + 0.44 and 6.23 + 0.45 respectively). Predation by seven spotted beetle upon different developmental stages of mealybugs was also studied. During the first hour of feeding, crawlers (1 st instar) were preferred over 2<sup>nd</sup> and third instar mealybugs. Beetles consumed more mealybugs when kept starved but this preference was only for crawlers (Means + SE = 25.60 + 0.39 (starved beetles), 16.82 + 0.37 (un starved beetles). This study highlights that feeding potential of predatory seven spotted ladybird is not only affected by the type of host but also the condition of host (live vs frozen). Moreover, starvation level of the predator can also be an important factor in determining its devouring capacity.

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#### **SCREENING OF BRINJAL VARIETIES FOR RESISTANCE AGAINST COTTON APHID (*APHIS GOSSYPHII* GLOVER)**

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Brinjal (*Solanum melongena* L.) is an important solanaceous vegetable crop of sub-tropics and tropics. It is extensively grown in our country. The plant is attacked by both chewing and sucking pests and cotton aphid (*Aphis gossypii* Glover) is an important sucking pest. Chemical control is widely used for the management of insect pests in brinjal, which causes a number of environmental problems. Resistant and tolerant varieties form the basic component of Integrated Pest Management (IPM). The present study was carried out at Sahiwal to screen different varieties of brinjal to identify tolerant variety/ varieties. The varieties tested were Nirala, Dilnasheen, Round Black, Black Beauty and Bemisal. Nursery was grown in pots and crop was transplanted in the field in mid February during 2012. The experiment was laid out in a Randomized Complete Block Design with four replications. There were four rows of 5.0 m length in each plot. Row to row spacing was 1.0 m and plant to plant distance of 30.0 cm. Aphid numbers (adults+ nymphs) were recorded from three randomly selected plants in each row. From each plant aphids were counted on the underside of three leaves by selecting one leaf from the top one third, one from the middle one third and one from the bottom one third. Data were recorded at weekly intervals from March 12 to April 9. Variety Bemisal had highest and Dianasheen had lowest number of aphid per leaf on most of the sampling dates. Seasonal mean number of aphids was also highest (6.7) on the variety Bemisal and lowest (5.5) on Dilnasheen. It is recommended that the variety Dilnasheen be used in IPM program for aphid management.

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### IMPACT OF ELEVATED CO<sub>2</sub> BACKGROUND LEVELS ON THE HOST-SEEKING BEHAVIOUR OF *AEDES AEGYPTI*

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Mosquitoes rely on carbon dioxide (CO<sub>2</sub>) to detect and orient towards their blood hosts. However, the variable and rapid fluctuations of atmospheric CO<sub>2</sub> concentrations may impact the host-seeking behaviour of mosquitoes. In this study, we analysed the effect of transient elevated background levels of CO<sub>2</sub> on the host-seeking behaviour and the physiological characteristics of the CO<sub>2</sub>-sensitive olfactory receptor neurones (ORNs) in female yellow fever mosquitoes, *Aedes aegypti*. We show that the take off and source contact behaviour of *Ae. aegypti* is impeded at elevated background levels of CO<sub>2</sub> as a result of masking of the stimulus signal. The mechanism underlying this masking during take off behaviour is one of sensory constraint. We show that the net response of the CO<sub>2</sub>-ORNs regulates this CO<sub>2</sub>-related behaviour. Since these neurones themselves are not habituated or fatigued by the transient elevation of background CO<sub>2</sub>, we propose that habituation of second order neurones in response to the elevated CO<sub>2</sub>-ORN activity could be one mechanism by which the net response is transduced by the olfactory system. The findings from this study may help to predict future shifts in mosquito-host interactions and consequently to predict vectorial capacity in the light of climate change.

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### TOXICITY OF CONVENTIONAL AND NEW CHEMICAL INSECTICIDES TO DUSKY COTTON BUG *OXYCARENUS LAETUS* KIRBY (HEMIPTERA: LYGAEIDAE)

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*Oxycarenus* spp. has become one of the serious global pests of family Malvaceae, vegetables, and fruits. In Pakistan, Dusky cotton bug, *Oxycarenus laetus* (Kirby) causes both qualitative and quantitative losses in cotton. Toxicity of four conventional and six new chemical insecticides was evaluated by leaf dip bioassay on the field population of *O. laetus*. So, a work regarding its toxicity was done in the insecticides resistance laboratory (Faculty of Agricultural Sciences and Technology) B.Z.U. Multan. Data regarding conventional insecticides and new chemistry insecticides were taken after 48 hours and 72 hours, respectively. Among conventional insecticides tested, a more toxic insecticide was profenofos. Toxicity order from highest to lowest in the remaining

conventional insecticides was: triazophos > bifenthrin > cypermethrin. Among new chemical insecticides tested, a more toxic was chlorfenapyr. The order of toxicities from highest to lowest in the remaining new chemical insecticides was: emamectin benzoate > imidacloprid > abamectin > spinosad > spirotetramat. So, profenofos and chlorfenapyr showed more effectiveness against *O. laetus* with their lower LC<sub>50</sub> values of 18.551ppm and 2.69 ppm, respectively. This study gave the opportunity to compare among the toxicity of conventional and new chemical insecticides and will be helpful in the integrated pest management program of *O. laetus*.

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### LARVICIDAL EFFICACY OF *OCIMUM BASILICUM* AND *MENTHA PIPERITA* AGAINST *CULEX QUINQUEFASCIATUS*

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The aim of this study was to investigate the Larvicidal Efficacy of *Ocimum basilicum* and *Mentha piperita* against *Culex quinquefasciatus*. Mosquitoes remain the important group of insects in terms of public health importance. They spread a number of diseases such as filariasis, malaria, Japanese encephalitis etc., causing millions of death every year. Natural products of plant sources with insecticidal properties have been used for control of a diversity of pest insects and vectors. The present study is centered on evaluation of the larvicidal activity to determine the efficacies of water and ethanol leaf extracts of *Mentha piperita* and *Ocimum basilicum* medicinal plants tested against 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar larvae of malaria vector in birds, lymphatic filariasis *Culex quinquefasciatus* (earlier name: *Culex fatigans*). The larvicidal activity was determined against *C. quinquefasciatus* in various concentrations (80 ppm, 160 ppm, 240 ppm, 320 ppm, 400 ppm) under laboratory conditions. Larvae were exposed to a range of concentrations of each extract. The larval mortality was assessed after 12, 24, 36 and 48 hrs. exposure and LC<sub>50</sub> and LC<sub>90</sub> were calculated for each time interval. Among two solvent extracts tested the maximum efficacy was observed in the ethanol extracts of leaves of *M. piperita*. 0 mortality was observed in control. Protein (mg/g), Carbohydrate (mg/g) and lipid mg/dl (triglyceride, cholesterol and high density lipid) were estimated by Lowry's method, phenol Sulphuric acid method and by biochemistry analyzer. These plants extracts are therefore promising as an alternative to synthetic insecticide mosquito control programmed. Thus providing the basis to use the plant extract against *C. quinquefasciatus* (*Culex fatigans*). It can be concluded from the present study that the ethanolic extracts of *M. piperita* possess active compounds for the development of larvicidal activity

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**EFFECT OF DIFFERENT REARING DIETS ON THE BIOTIC POTENTIAL OF  
INDIAN MEAL MOTH *PLODIA INTERPUNCTELLA* (HUBNER)  
(LEPIDOPTERA: PYRALIDAE) UNDER LABORATORY CONDITIONS.**

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*Plodia interpunctella* (Lepidoptera: Pyralidae) is an important pest of stored commodities. It infests variety of stored products and can survive under diverse climatic conditions. Being multivoltine it can be used as a fictitious host for the rearing of bio-control agents. The studies were conducted to determine the biotic potential of *P. interpunctella* on various diets. Significant difference was recorded among the tested diets for biological parameters of *P. intrepunctella*. It was found that diet based on maize was superior to wheat, oat and wheat bran because it produced maximum adults with minimum developmental time and maximum number of females with better egg laying response. Fecundity was minimum (Mean  $\pm$ SE 85.16  $\pm$ .21) for wheat bran whereas, it was maximum (138.33 $\pm$ 2.87) for oatmeal diet. Maximum number of female (17.66  $\pm$ 1.38) was recorded for maize flour diet however; it was minimum for the oatmeal (5.33 $\pm$ 1.07). Duration for completion of the life cycle was also affected by the type of diet offered during immature developmental stage. Average developmental period recorded for maize (27.74 $\pm$ 0.28) was shorter than wheat bran diet (34.88 $\pm$ 0.44).

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**INTEGRATED PEST MANAGEMENT OF BRINJAL FRUIT BORER  
(LEOCINODES ORBONALIS GUEN.): AN OVERVIEW**

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Brinjal (*Solanum melongena* L.) is an important vegetable and is grown in Southeast Asia, Middle East and Europe. A number of insect pests feed on this vegetable, and Brinjal Fruit Borer is one of the most important insect pests. It attacks all parts of brinjal plant except roots, but fruits and growing shoots are severely damaged. It bores into tender shoots and after flowering, moves to flowers and finally to fruit. This insect pest causes up to 70 to 92 percent losses. Farmers have been relying heavily on pesticide application to control this pest. Extensive use of pesticides have resulted in low profitability for producers, increase in price for the consumers and caused environmental problems. Integrated pest management (IPM) is an effective and environmentally sensitive approach to pest management that relies on combination of a number of pest control practices. IPM is not about eliminating the pest, but the aim of IPM is to reduce

pest populations to avoid damage levels that cause yield losses. IPM strategies may be different for each crop, country or the region. Therefore, it cannot be delivered as a package but have to be developed to fit local requirements. An IMP strategy based on sanitation, withholding pesticide application as long as possible, using resistant varieties and pheromone traps have been successfully applied in many areas of the Southeast Asia. The same techniques need to be field tested and promoted in Pakistan.

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### **EFFECT OF SOME INSECTICIDES ON DAMAGE COMPENSATION IN COTTON**

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The study was conducted to determine the effect of insecticides on damage compensation of cotton under field conditions at Latif farm, SAU Tando Jam during 2007-2008. The cotton variety Chris 134 was sown in 3<sup>rd</sup> week of May by dibbling method on furrows. The experiment was laid out in randomized complete block design with seven treatments. Pest damage was simulated by artificially removing the leaves and fruiting bodies of cotton plant. The leaves and fruiting bodies removal ranged between 15 and 75%. Two controls (checks) were maintained, one (T1) in which leaves and fruiting bodies were not removed and insecticides were also not applied and another (T7) in which leaves and fruiting bodies were not removed but Insecticides were applied. Two applications of Nitrophos fertilizer at recommended doses were applied, 64 and 88 days after sowing. Four Insecticides viz., Confidor, Mospilan, Deltaphos and Tracer were sprayed twice at recommended doses with knapsack sprayer. The observations on sucking complex such as, thrips, *Scirtothrips dorsalis* Hood, white fly, *Bemisia tabaci* Lind, and jassid, *Amrasca devastans* Dist. were recorded. The observations on bollworms were also recorded at the time of attack. The volume of cotton bolls were measured with the help of vernier caliper. The crop maturity was observed by the opening of the bolls. The results indicated that there was no effect of removal of leaves and fruiting bodies, and application of insecticides on infestation of thrips, whitefly, jassid and infestation of bollworms compared to control plot. There was no significant difference of plant height in different treatments. The analysis of results indicated that there was a significant difference ( $P < 0.05$ ) among yield of different treatments.

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### **PARASITIZATION OF *PLUTELLA XYLOSTELLA* (L.) ON CAULIFLOWER CROP IN SOUTHERN PAKISTAN**

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Larvae of diamondback moth (DBM), *Plutella xylostella* (L.) cause severe economic loss to brassica vegetable crops in Pakistan. Studies were carried out to identify

the indigenous parasitoids attacking diamondback moth and to assess their relative importance for population management of *P. xylostella*. Six parasitoid species were collected and identified attacking larvae and pupae of *P. xylostella*. These included the larval parasitoid *Cotesia plutellae* kurdjumov (Braconidae), *Cotesia* sp., *Diadegma* sp. (Ichneumonidae), a larval-pupal parasitoid *Oomyzus sokolowskii* kurdjumov (Eulophidae), a pupal parasitoid *Diadromus collaris* Gravenhorst (Ichneumonidae) and a pupal parasitoid *Brachymeria apanteles* Risbec (Chalcididae). *C. plutellae* was the dominant larval parasitoid followed by *O. sokolowskii* in their abundance and parasitism under cauliflower fields. Their average parasitism ranged from 2.56 to 61.97% and 1.33 to 13.10%, respectively. The average parasitism caused by *Cotesia* sp. was less than three percent. The remaining parasitoids were scarce and caused less than 0.5% mortality each on an average. Further studies on parasitism characteristics of *O. sokolowskii* showed that there was a significant ( $P < 0.01$ ) positive correlation between *P. xylostella* pupal weight and the number of parasitoids emerging from a pupa. More than 50% of the parasitized pupae of *P. xylostella* supported 6-10 *O. sokolowskii* adults per pupa.

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**EFFECT OF TEN DIFFERENT PLANTS AND DELTAMETHRIN AT  
DIFFERENT CONCENTRATIONS ON *TRIBOLIUM CASTANEUM* (HERBST)  
UNDER LABORATORY CONDITIONS**

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Deltamethrin and ten different plants (powders) were tested on the pupae and adults of *Tribolium castaneum* (Herbst) at the Eco-Toxicology Laboratory of the Department of Entomology, BZU Multan. Four concentrations were made for each plant powder and deltamethrin was tested as standard. Each concentration was mixed in the 60 grams of wheat flour. Results showed that minimum number of adults (0.16) were recorded in wheat flour treated with neem seed powder whereas minimum number of pupae (14.38) was recorded in wheat flour treated with castor beans powder. Different concentrations of the different plant showed significant differences among them for the pupae and adults of *T. castaneum*. Neem seed powder was found very effective for adults and pupae of *T. castaneum* in all the four concentrations. Deltamethrin also gave significant results and proved very effective against *T. castaneum* adults and pupae. Reasonably low numbers of adults and pupae (1.85 and 7.70) survived in deltamethrin treatment.

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**ADULT OVIPOSITION PREFERENCE AND OFFSPRING PERFORMANCE OF  
FRUIT FLY, *BACTROCERA ZONATA* (DIPTERA: TEPHRITIDAE) ON  
DIFFERENT RIPENING STAGES OF MANGO *MANGIFERA INDICA* L. FRUIT**

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The best way to avoid damage problems due to fruit flies is to eliminate their sources of attraction because they are more attracted to ripened fruits and vegetables. An evaluation of fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) adult oviposition preference and offspring performance was conducted to observe the effects of different fruit ripening stages of two mango *Mangifera indica* L. varieties (Desi and Malda). The mango fruits were taken from local Establishment at unripe (hard-green), ripe (green-yellow) and fully-ripe (yellow) stages. Fruit attributes of both mango varieties at three stages of ripeness were the mainly noticeable factors effecting adult response and larval performance in the experiments. Female *B. zonata* exhibited an elevated oviposition preference to ripe and fully-ripe mango fruits, while responded less to unripe fruit of both varieties. Green color of unripe mango was probably less attractive to *B. zonata* flies over ripe (green-yellow) and fully-ripe (yellow) fruits. Additionally, greater rate of *B. zonata* larval survival and short development time in ripe fruit corresponded to preference of female flies to ripe fruit of mango varieties. The relationships between host preference of female flies, and pericarp toughness and total soluble solids of mango, showed less attractiveness of flies to fruits with higher pericarp toughness and lower total soluble solids. Nevertheless, if green or green-yellow fruits are harvested, field infestation rates by fruit flies would be especially minimum thus appearing to be a successful methodology and ought to prove useful tool in further efforts of pest control.

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**INTEGRATED PEST MANAGEMENT IN ORGANIC COTTON**

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Studies were carried out to evaluate the effect of organic cotton production system on insect pests of cotton. There were five treatments including control. The treatments comprise: Farm yard manure, poultry manure, green manuring with dhancha, conventional integrated pest management and control, *i.e.* without any use of agrochemicals and interventions. When pest population approached economic injury level, neem kernel oil was applied in organic treatments and Radiant® was applied in conventional IPM treatment. The maximum thrip, *Scirptothrips dorsalis* population (3.43 insects/leaf) was recorded in poultry manure treatment followed by farm yard manure

treatment. The minimum pest population was recorded in IPM treatment (2.06insects/leaf). The same trend of pest population growth was recorded in white fly, *Bemisia tabbaci* and jassid, *Amrascca bigutula bigutula*. The yield data indicated that there was a highly significant difference in yield of cotton of different treatments. The highest yield was obtained in farm yard manure treatment followed by IPM and poultry manure. There was no significant difference between green manure and control; however, yield of green manure was higher than control treatment.

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#### INCIDENCE OF INSECTICIDE RESISTANCE IN FIELD STRAINS OF *Aedes albopictus* IN KHYBER PAKHTUNKHWA

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Insecticides play a key role in the agricultural production and public health in Pakistan. Injudicious insecticidal application results in environmental and health hazards as well as development of resistance in the pests due to high selection pressure. This paper aims to assess resistance/susceptibility status in the field population of *Aedes albopictus*. Larvae/adults samples of *Ae. albopictus* were collected from various habitats at various locations with history of insecticides usage from four districts of Khyber Pakhtunkhwa and were tested *in vitro* against commonly used public health insecticides, viz., chlorpyrifos, deltamethrin, lambdacyhalothrin and temephos as per WHO protocols. The results indicated that the mortality range exhibited by chlorpyrifos and lambdacyhalothrin (30-80%) was comparatively low in mosquitoes indicating the resistance level of these insecticides against *Ae. spp.* in field strains collected from IFA, Torn, Malakadher and Hayatabad after 1, 24 and 48 h exposure period. While the mosquitoes in area with low selection pressure of insecticides i.e. Military farm Khaishk, Azakhel Park and Naguman were highly susceptible to the insecticides after 24 and 48 h exposure period. The application of deltamethrin resulted 100% mortality in field collected strains of Military dairy farm Khaishk, Azakhel Park and Naguman after 24 and 48 h exposure period similar to laboratory susceptible strain. Temephos @ recommended dose was found equally toxic (100%) at all sites against *Ae. spp.* except NIF A and Hayatabad. The toxicity range of insecticides observed was temephos > deltamethrin > lambdacyhalothrin > chlorpyrifos. The study recommends the regular monitoring of resistance levels of different insecticides against important vector species.

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**INTEGRATION OF NATURAL ENEMIES, MINERAL OIL AND INSECTICIDE  
AGAINST *PHYLLOCNISTIS CITRELLA* STANTON (LEPIDOPTERA:  
GRACILLARIIDAE) IN A CITRUS NURSERY OF FAISALABAD, PUNJAB,  
PAKISTAN**

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The citrus leaf miner (CLM) is a pest of regular occurrence in nurseries, young plantations and tender flushes of the citrus groves. It is most damaging in nursery considering the economic loss incurred and the havoc it plays if left unattended. Due to the posed hazards of pesticides, research to develop an integrated management program is necessary. An experiment was conducted in August in a citrus nursery. The present studies consisted of integration of releases of *Chrysoperla carnea* larvae, *Citrostichus phyllocnistoides* with application of Diver, a mineral oil and the insecticide, emamectin benzoate. The treatments in which only *Chrysoperla carnea* and *Citrostichus phyllocnistoides* were released, population per flush ranged from  $3.90 \pm 0.84$  to  $4.47 \pm 0.13$  and  $4.03 \pm 0.51$  to  $4.70 \pm 0.60$  respectively. The treatments in which Diver and Emamectin were applied only, population of the pest per flush ranged from  $3.03 \pm 0.28$  to  $4.20 \pm 0.36$  and  $3.53 \pm 0.51$  to  $4.68 \pm 0.60$  respectively. Among the Integrated measures applied, the most effective was *Chrysoperla*+Diver with  $0.33 \pm 0.27$  to  $3.43 \pm 1.58$  pest per flush followed by *Citrostichus* + Diver, *Chrysoperla* + Emamectin and *Citrostichus* + Emamectin with  $2.1 \pm 0.28$  to  $4.3 \pm 0.36$ ,  $3.23 \pm 0.32$  to  $4.27 \pm 1.02$  and  $3.5 \pm 0.23$  to  $4.7 \pm 0.24$  respectively. Result of repeated measure analysis revealed that treatment of 15 larvae of *Chrysoperla*+Diver at 0.15% and 10+0.1 resulted in 87% and 67% control of the pest population followed by *Chrysoperla*+Emamectin 15 + 0.15 and 10 + 0.1 with 50% pest control during the study period.

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**MANAGEMENT OF FRUIT FLY THROUGH BAIT APPLICATION  
TECHNIQUE (BAT) IN MANGO ORCHARDS**

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Mango fruit fly is an important insect pest of mango fruits worldwide. Different chemical and non-chemical methods are used for its management. Efficacy of bait application technique (BAT) by using different insecticides viz; Imidachloprid (Confidor 200SL), Spinosad (Tracer 240SC), Bifenthrin (Talstar 10EC) and Spirotetramat



(Movento 240SC) was studied when these were mixed with protein hydrolysate to conduct spot prays at fortnight intervals for the control of Fruit Fly on mango cultivar Chaunsa White under field conditions at Mango Research Institute Multan. Each insecticide with its recommended / calculated dose was mixed separately according to the percentage of 30ml protein hydrolysate solution in one liter of water. Fruit fly infestation was examined through observing twenty dropped fruits randomly under each experimental plant on weekly basis starting from marble stage of fruit development up to its harvest. Maximum infestation decrease percentage (79.35) was observed in T<sub>4</sub> where the experimental plants were sprayed with protein hydrolysate mixed with Spirotetramat followed by 59.70 percent in T<sub>1</sub> (protein hydrolysate + imidachloprid) while its minimum value was noted in T<sub>2</sub> where protein hydrolysate was sprayed with Spinosad. This proves that Spirotetramat mixed with protein hydrolysate is the best option as alternative control strategy for mango fruit fly.

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#### **EFFECTS OF EMAMECTIN BENZOATE INSECTICIDE ON THE WEB DESIGN OF *NEOSCONA THEISI***

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The insecticides used to control insect pests have direct (death) or indirect (changes in behaviour of physiology) effects on the populations of spiders. The changes in geometric patterns of web were evident even at low doses of pesticides. Sublethal effects of insecticides also include delay in web building and reduction in web size. The present study was conducted to investigate the effects of insecticide emamectin benzoate on survival and web parameters (mesh height, capture area, capture thread length and number of radii) of *Neoscona theisi*. For the study, different doses of insecticide were sprayed on the spiders using micropipette and housed in 3D frames (60x60 cm) to construct webs. Their effects were observed on survival, web appearance and web parameters at 24h. The calculated LC<sub>50</sub> was 434.24 nl/ml and decreased with the time. A significant difference in mesh height, capture area and capture thread length were recorded in the treated spiders as compared to control. Mesh height showed positive while capture area and capture thread length negative correlation with the concentration of insecticide. At LC<sub>50</sub>, the spiders formed abnormal webs. However, with the passage of time the most of the spiders recovered of their normal web building behaviour. It was concluded that the sublethal doses of insecticides affected the web building behaviour of surviving spiders for a specific period.

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**STUDIES ON THE EFFECT OF RADIATION ON OPTIMUM POPULATION  
DENSITY OF *PLODIA INTERPUNCTELLA* (HUBNER) (LEPIDOPTERA:  
PYRALIDAE)**

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Stored food stuffs are infested by insect pests and the Indian meal moth is the most important pest of stored products. Present study was designed to assess detrimental effect of irradiation on developmental stages of Indian meal moth. Eggs of *P. interpunctella* have been treated by  $\gamma$  radiation from Cesium-137 source from 5-15Gy and studied with control. Thus it may be concluded that the radiation significantly affects the parent generation, and does not produce any detrimental effect on the development of the parent. The percent viability and percent successful completion of development in parent generation are comparatively high. Results have shown that radiation affects the biological parameters such as: Adult emergence, larval and pupal duration, male-female sex-ratio and fecundity of this pest. In parents it has been observed that adult emergence found to be 80%, 20%, 40% and 50% in control, 5 Gy, 10 Gy and 15 Gy, respectively, whereas, in F1 is about 85%, 2%, 10% and 30% in the control and radiation doses of 5Gy, 10 Gy and 15 Gy. Population suppression has been recorded in parent to F1.

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**SCREENING FOR BRUCHIDS (*CALLOSOBRUCHUS MACULATUS*)  
TOLERANCE IN MUNGBEAN GENOTYPES UNDER LAB CONDITION**

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The entomological research on bruchids is aimed at screening progenies obtained from crosses made by Mungbean Breeder for resistance/tolerance to beetles. Pulse beetle (*Callosobruchus maculatus*) (F.) are the principal post-harvest pest of mungbean and other stored pulses. 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. Bruchids infestation causes reductions in the weight, seed viability, sale ability and infested grains unfit for human consumption. The alternative to chemicals and other control measures is to develop bruchids resistant genotypes required for storage purposes. Culture of bruchids beetle was maintained on bold mungbean grains at  $28\pm 2^{\circ}\text{C}$  and  $70\pm 5\%$  relative humidity. Insect of uniform age males and females were collected separately by isolating mungbean grains in small

transparent glass test tubes mouth plugged with cotton. 10-15 pairs of newly emerged adults were collected within 24 hours and released in glass/plastic jars containing sound grains of mungbean. The jars were covered with muslin cloth to facilitate aeration. The stock culture maintained was utilized for conducting the experiment. 28 mungbean crossed genotypes were evaluated to ascertain their resistance to bruchid beetles. The resistance of mungbean genotypes to bruchids was evaluated on the basis of, adult population developed from eggs laid, grain infestation, developmental period and % grain damage. The results indicated that none of the tested genotypes was completely resistant to bruchid attack. Out of 28 genotypes, one genotype *i.e.* 2802 X Ramazan (22-10) had the lowest grain damage *i.e.* 20.93% indicated tolerant to bruchids. Maximum grain damage 91.77% was recorded in genotype 2809 X Ramazan (22-9) with maximum and adult progeny. The remaining genotypes indicated susceptibility to bruchids.

**PREDATORY EFFICIENCY OF *OXYOPES JAVANUS* AGAINST WHITE-BACKED BROWN PLANTHOPPER (*SOGATELLA FURCIFERA*)**

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Functional response is one of the most important behavioral characteristics that reveal different aspects of prey-predator interactions. In this study, functional response of *Oxyopes javanus* (Araneae: Oxyopidae) against white-backed brown planthopper (*Sogatella furcifera*) was evaluated in laboratory and microcosm. For this purpose, adults of predator were presented to different densities (5, 10, 15, 20, 30 and 40) of *S. furcifera* and mortality recorded after 48 hours. Logistic regression analysis determine of type II functional response for adult *O. javanus* both in lab and microcosm. Nonlinear least-square regression was used to estimate the attack rate ( $a$ ) and handling time ( $T_h$ ) of the predator. The attack rate and efficiency of attack per hour was high in lab (0.50 and 0.34 respectively) as compared to microcosm (0.42 and 0.11 respectively). However, handling time was more in microcosm (3.66 per hour) as compared to lab (1.44 per hour) due to complexity of habitat. The observed feeding strategy of *O. javanus* suggested that spiders have positive role in controlling agricultural pest such as white-backed brown planthopper in a density sensitive way.

**EFFECT OF MULTIPLE PREDATORS ON THE SUPPRESSION OF APHID IN LABORATORY AND MICROCOSM**

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Interaction of predators may relax the prey population from predation pressure.

The rate at which predator kills its prey at different densities is called functional response. It provides the foundation of predator prey interaction. The present study was conducted to investigate the presence of two predators' viz., *Oxyopes javanus* and *Coccinella septumpunctata* against four different densities of prey, *Sitobiona avenae*, in laboratory and microcosm. Both in laboratory and microcosm experiment *C. septumpunctata* larvae respond maximally followed by *C. septumpunctata* adult and *O. javanus* in the consumption of aphids. To check antagonistic effect of predators, aphid were offered to combination of predators i.e. *C. septumpunctata* larvae + *O. javanus* and *C. septumpunctata* adult + *O. javanus*. The consumption rate was low in the presence of both predators as compare to their individual consumption rate both in laboratory and microcosm. Consumption rate of *C. septumpunctata* adult + *O. javanus* was higher than *C. septumpunctata* larvae + *O. javanus* in microcosm. However, in laboratory both combinations have equal consumption rate. The consumption rate of all predators and their combinations against *S. avenae* was higher in laboratory as compare to microcosm. It can be concluded from the study that presence of both predators increase the survival of the aphid population.

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**LABORATORY EVALUATION OF CHEMICAL AND BIOLOGICAL  
INSECTICIDES AGAINST 4TH INSTAR LARVAE OF CULEX  
QUINQUEFASCIATUS (DIPTERA: CULICIDAE)**

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Present study was conducted to isolate *Bacillus thuringiensis* var. *israelensis* (*Bti*) from soil samples taken from different localities and to determine its efficacy against 4<sup>th</sup> instar larvae of *Culex quinquefasciatus*. The effectiveness of temephos (trade name: Relief T) against the 4<sup>th</sup> instar larvae of *Culex quinquefasciatus* was also determined. The efficacy of turpentine oil with *Bti* and temephos was also compared. Larvae of *Culex* mosquitoes were reared in laboratory under optimal conditions. *Bacillus thuringiensis* is Gram- positive, spore- forming bacteria, used as a larvicide against many Dipteran species due to the cry toxins produced by it. 26 soil samples were taken, out of which 5 showed positive results. In laboratory assays, 100µl toxins was applied in each beaker (no. of larvae in each beaker= 10). *Bti* was applied for 2 hours and mortality rate was recorded after every 30 minutes. The efficacy of *Bti* against larvae was significant  $P < 0.05$ ,  $df = 5, 6$  and  $F = 10.8$ . In laboratory assay of temephos, 0.15g temephos was dissolved in 1 liter distilled water and 4<sup>th</sup> instar larvae ( $n = 30$ ) were introduced in replicates and in control group. The efficacy of temephos against *Culex* larvae was significant ( $df = 12, 26$ ;  $F = 29.6$ ;  $p < 0.05$ ). 100µl solution of turpentine oil along with *Bti* was applied on *Culex* larvae. The efficacy of turpentine oil with *Bti* was significant ( $df = 1, 2$ ;  $F = 49.0$ ;  $p < 0.05$ ). Turpentine oil and *Bti* showed highest mortality rate in comparison with temephos.

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**INCIDENCE AND CONTROL OF PEACH FLAT-HEADED BORER,  
*SPHENOPTERA DADKHANI* (OBN.) DAMAGING TREES OF STONE FRUIT  
ORCHARDS**

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*NIFA, Tarnab, Peshawar*

Peach flat-headed-borer, *Sphenoptera dadkhani* (Oben.) is the major insect pest of stone fruit. During survey, Peach flat-headed borer infestation (70-90 %) was observed in stone fruit orchards of Nowshera, Peshawar & Swat. Development of Degree Day model: Adult emergence started in the first week of April (CDD=567-573). CDD summations to first emergence in the 2<sup>nd</sup> generation ranged from 2355-2374 and lasted till the second week of August. Third generation emergence was observed in October-August when 4545-4580 DD were accumulated. Two sprays of Thiodan (Endosulfan), Confidor (imidacloprid) or Fyfenon (Malathion) were more effective in reducing the damage at the time of first adult emergence (when 570 DD were accumulated). Different pesticides in Bordeaux mixture like (Nurelle-D & Thiodan @ 75 ml each were effective in combination with (75 ml COC + 3 kg Lime + 10 L of water) against borer in the month of January in plum orchard.

**STAGE SPECIFIC LIFE TABLE OF MEALYBUG, *PHENACOCCLUS*  
*SOLENOPSIS* (TINSLEY) UNDER COTTON FIELD CONDITIONS**

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The field study was conducted on Stage specific life table studies of mealybug, *Phenacoccus solenopsis* (Tinsley) under cotton field conditions at the experimental field of Sindh Agriculture University Tandojam during, 2013. Two hundred eggs were kept for each replication on cotton plants grown in large pots. When eggs hatched, the life table parameters such as, apparent mortality, survival fraction, mortality survivor ratio, indispensable mortality and k-value were studied. Natural enemies such as *Chrysoperla carnea*, lady bird beetles, Spiders and *Anasius bambawallae* were found active on the potted and surrounding cotton plants in the field conditions. The total mortality in egg stage went to 19.00%. The mortality percentages of 1<sup>st</sup> instars due to natural enemies recorded was (18.73%). While, in 2<sup>nd</sup> instars (13.13%) mortality was recorded. The total combined mortality in 1<sup>st</sup> and 2<sup>nd</sup> instars recorded was (26.34%) and (15.64%), respectively. However, the mortality percentages of 3<sup>rd</sup> instars and pupae due to parasitoids were recorded as (7.28%). The total mortality (10.26%) was recorded in 3<sup>rd</sup> instars / pupae. The total mortality percentage (54.83%) in cotton potted plants was recorded. Consequently, (45.17%) of adults survived with male and female ratio as ♂1:

6.52♀, respectively. The results further indicated that the highest apparent mortality was (26.34%), indispensable mortality (96.89) and k-value (0.13) were recorded in 1<sup>st</sup> instar. The maximum survival fraction was recorded as (0.90) in third instars/pupae. The total k-value generations (0.35) was recorded on cotton mealybugs. The study revealed that the predators and parasitoids should be encouraged in cotton field when mealybugs appear, because predators are highly voracious feeder of 1<sup>st</sup> and 2<sup>nd</sup> mealybug instars.

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### ENVIRONMENT FRIENDLY CONTROL TECHNIQUES FOR MOSQUITOES

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Oils from Cinnamon *Cinnamomum zeylanicum*, Canola *Brassica napus*, Clove *Syzygium aromaticum*, Eucalyptus *Eucalyptus grandis*, pine resin *Pinus sylvestris* (turpentine), mustard *Brassica Juncea*, neem *Azadirachta indica*, and kerosene were tested for their mortality on early 4<sup>th</sup> instar larvae of *Culex quinquefasciatus* Say and in field ovitraps for their repellency/attractancy. The 24 h LC<sub>50</sub> for these oils as recorded (in ppm) were; eucalyptus 166 (66.4), canola 675, cinnamon 429 (107.25), clove 510, mustard 2599, neem 642.32, turpentine 199, and kerosene 112. The LC<sub>90</sub> values (ppm) at the 24 h post application time were eucalyptus 459 (183), canola 3453, cinnamon 2538 (634.5), clove 12246, mustard 9131, neem 1847, turpentine 492, and kerosene 219. The 48 hrs LC<sub>50</sub> for these oils as recorded (ppm) were eucalyptus 62.0, canola 321, cinnamon 46.56, clove 211, mustard 694, neem 475.84, turpentine 125, and kerosene 105 respectively while the LC<sub>90</sub> values at 48 h post observation time were eucalyptus 191.6, canola 1372, cinnamon 281, clove 865, mustard 1832, neem 1035.48, turpentine 254, and kerosene 207. The 24 and 48 h LC<sub>50</sub> values for eucalyptus, cinnamon, turpentine and kerosene oils did not differ at p<0.05 while the 24 h LC<sub>90</sub> of eucalyptus and turpentine were at par and the 48 h LC<sub>90</sub> for turpentine and kerosene were statistically the same indicating equal toxicity to the *Cx. quinquefasciatus* mosquitoes. The positive oviposition activity index (OVI) for traps treated with eucalyptus, cinnamon, turpentine and kerosene oils indicated that these oils were attractive while neem oil was repellent to *Aedes albopictus* mosquitoes.

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### USING BORIC ACID AS A SLOW-ACTING TOXICANT AGAINST SUBTERRANEAN TERMITES

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Various concentrations (100, 500, 1000, 5000 and 10000 ppm) of boric acid were

tested to see its potential as a slow-acting toxicant against subterranean termite, *Heterotermes indicola* (Rhinotermitidae: Isoptera). Under the tested concentrations, ELT 50 values ranged from 2.7 to 5.4 days while ELT 90 were 4.5 and 9.1 days for the lowest and highest concentrations used in the trial. Choice feeding tested showed that food treated with all the above concentrations was acceptable to termites. At a concentration of 10000 ppm boric acid resulted in 90% mortality within two weeks when both the treated and untreated substrates were offered to *H. indicola*. Field testing of boric acid bait showed that it was readily fed upon by *Odontotermes* spp. and *Microtermes* spp. while *H. indicola* shied away from it after initial feeding. However, it led to significant population suppression for all the three genera of subterranean termites.

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**RELATION BETWEEN METALS (Cu & Cr) AND DETOXIFYING ENZYMES  
IN SPIDERS (ARANEAE: LYCOSIDAE) FROM POLLUTED AREAS**

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Chronic exposure to metals pollution can induce biochemical defensive mechanisms in spiders enabling them to survive additional stress. In view of that spiders can be used as bioindicator of heavy metal pollution in terrestrial ecosystem. Present study was conducted to evaluate the role of detoxifying enzymes viz. glutathione s-transferase (GST), acetylcholine esterase (AChE), carboxylesterase (CarE) and cytochrome oxidase in two spider species viz. *Pardosa oakleyi* and *Lycosa terrestris* exposed against heavy metals (Cu and Cr). For this purpose, spiders were collected from five sites i.e. adjacent area of Hudara drain, River Ravi, Thoker, Muhlanwal and from fallow land of University of the Punjab, Lahore by visual research method. Soil samples were also collected from same areas to find correlation between the quantity of heavy metals in soil and detoxifying enzymes of spiders. Concentration of Cu and Cr was highest at the site of River Ravi followed by Muhlanwal, Thoker, Hudara drain and Punjab University respectively. In both spider species, GST level shows positive correlation with the concentration of heavy metals. However, the level of Cytochrome oxidase did not varied in both spider species with the concentration of both metals. The quantity of AChE showed no correlation with the heavy metals in *P. oakleyi* and positive correlation in *L. terrestris*. In both spider species CarE have positive correlation with metals but correlation was not significant with Cr. This showed that quantity of detoxifying enzymes particularly GST in the spiders can be used as indicator of environmental pollution.

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### **ROLE OF SOME EXTENSION TOOLS IN SUCCESS OF RODENT PEST MANAGEMENT CAMPAIGN IN SINDH**

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Rodent pests cause immense losses to standing crops stored food and transmit many fatal diseases to human and livestock. According to recent survey, overall damage crops and stored commodity amounts to 2,540 million rupee annually. Pakistan has been gifted with different agro ecological zones due to variation in altitude, temperatures and rainfall etc resulting in occurrence of number of rat species. Among Field crops, wheat, paddy and sugarcane are worst effected crops and losses are estimated in millions rupees only. To enhance awareness level among farming community, a comprehensive study was conducted to compare the impact of audiovisual tools, *i.e.* documentary films, literature and lectures in farmer's gatherings. During Fy. 2010-13, 45 farmers' awareness campaigns were launched in which 2540 farmers and NGO's personnel were educated to apply latest developed PARC Rat bait packages to minimize rat damage to crops and other commodities. Post-awareness results of campaign indicated 25-30% increase in awareness level to adopt modern rat control technology. Mass media campaign coupled with on field demonstration is recommended to minimize rodent losses thus to enhance crop productivity.

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### **TAXONOMIC STUDIES OF RAT SPECIES INFESTING DATE-PALM ORCHORDS AT NOK-KUNDI, BALUCHISTAN**

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The rodent pest species live trapped from Nok-Kundi resembles to lessor bandicoot rat (*Bandicota bengalensis*) and short tailed mole rat (*Nesokia indica*). Some anatomical and behavioural differences were recorded in this rat species. This species makes long, branched and complicated tunnels from one tree to another tree just beneath about one ft under the surface of the ground measuring up to 30 m or more. The soil texture of the area is loose sandy and inner surface of soil under one feet is damp due to seepage water and relatively more compact enabling rats to burrow and live inside. Some prominent features of the rat species recorded are as follows: Male were heavier and weighed up to 496 g, while maximum weight of female was recorded 339g (ii) Tail size was small like *N. indica* but tail circumference was like *B. bengalensis* (iii) Guard hairs were present like *B. bengalensis* (iv) Colour of teeth was dark yellow like *B. bengalensis*,



(v) The species is semi fossorial, while *N. indica* is fossorial in habit (vi) The litter size was recorded 1-5 young ones like *N. indica* (vii) The species of rat is excellent climber like roof rat (*Rattus rattus*) and may climb on the crown of tree for the feeding of date fruit, (viii) Ear resemble to *B. bengalensis* in shape and size. (ix) Eyes were small and open in young ones after 21 days, while in *N. indica*, its open in 16-18 days. (x) The species is not aggressive, while *Bandicota* is an aggressive rat. (xi) The species has high cannibalistic behaviour like *B. bengalensis*, while cannibalism in *N.indica* is very low and mother does not react adversely to the pups. On handling to young ones this species eats/kill them or leaves the nursing like *B. bengalensis*. Taxonomical studies were conducted to determine the status of the rat species through reference collection of rodents in the Museum of Natural History, London. On the basis of shorter tail relative to head and body length (respectively 45% and 61% in these specimens, 75-80% in *Bandicota*) and shorter palatal foramina length « 5.00 in species under study, >8.00 in *Bandicota*). Most specimens of *Nesokia* in the collection do have paler incisors; however there are some specimens with similarly coloured incisors. In view of the above observation it was revealed that the specimens of the rodent are *Nesokia indicia* not *Bandicota*.

#### **FUNGAL CONTROL OF A SUBTERRANEAN TERMITE (*HETEROTERMES INDICOLA*)**

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Study was conducted for the isolation and identification of fungal species from cadavers of notorious subterranean termite, *Heterotermes indicola*. Virulency of *Rhizopus stolonifer* isolate and resultant variation in food consumption of *H indicola* workers was evaluated over a period (month) utilizing surface culture method. For different time exposure *R. stolonifer* was significantly able to produce epizootics on tested insect whereas food consumption was reduced. Behavioral change was observed as lethargic workers were eaten by other workers. Experiments revealed *R. stolonifer* is a potent repellent against *H indicola*.

#### **SYNERGISTIC INTERACTION BETWEEN CULTURE AND EXTRACTS OF NATURAL HOST PLANTS ON TRAPPING OF MELON FLY**

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Fruit flies (*Diptera, Tephridae*) cause large losses to various fruits and vegetables through the world and are recognized as major insect pests of horticultural industries. Pests and pesticides free supply of the produce has further increased their significance as

a requirement of WTO. Fruit flies can be controlled by regular application of insecticide sprays however this method is dangerous for bio-control agents, environment, beneficial insects and health hazards. Male annihilation using lure baited traps is a good alternative of pesticide spray for the control of male fruit flies. Baits containing attractants and toxicant are used for both sexes of the pests. The objective of the study was to assess the effectiveness of Cue-lure mixed with natural fruit extract against melon fruit fly, *Bactrocera cucurbitae* under field conditions. For this purpose extract of melon, sponge gourd, bitter gourd and round gourd were mixed in Cue-lure. Diptrex as killing agent was added @ 5% in all the test materials. Twenty milliliter of the test mixture per trap was applied on cotton wicks put inside the trap. Each treatment was replicated four times. Field experiment was conducted, installing traps in bitter gourd field in randomized design at Peshawar. Fruit fly captured/ traps were recorded weekly for 10 weeks and the treatments effect was evaluated by assessing adult flies captured/ trap. The results showed that maximum number of 944 flies were captured in trap loaded with cue-lure and sponge gourd followed by 793, 787.5 and 725.3 flies in cue -lure combination with melon, bitter gourd and round gourd respectively. Cue-lure standard trap caught 305.8 flies.

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#### **IMPACT OF ORGANOPHOSPHATE PESTICIDES, CHLORPYRIFOS AND MALATHION ON GREEN MUSSELS (*PERNA VIRIDIS*)**

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Pesticides are used widely for the control of pest in agriculture to increase the yield of crop. These pesticides can enter the marine ecosystem through different sources and cause lethal and sublethal effects on marine organisms. The objective of the present work was to study the effect of organophosphorus pesticides (chlorpyrifos and malathion) on protein contents of different tissues for *e.g.* mantle, adductor muscle and gills of *Perna viridis* (green mussels). Mussels were exposed to 0.5, 1.5, 2.5 and 3.5 ppm of chlorpyrifos and malathion for 48 and 96 h, separately. There was significant decrease in protein contents in tissues as compared to control values with gills as the most affected tissue. The percent decrease in protein contents was in the order of: gill > adductor muscle > mantle. The effect of both pesticides was concentration as well as time dependent. The results obtained showed that chlorpyrifos is more toxic to *Perna viridis* as compared to malathion.

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**STUDY ON THE PARASITISM OF GREEN STINK BUG (PENTATOMIDAE)  
FROM PAKISTAN**

NAILA

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The survey of parasites of green stink bugs was conducted during the year 2012 - 2013. Green stink bug, (*C. hilare*) is a destructive agricultural pest in Pakistan especially feeding on valuable crops such as vegetables, grains, fruits, nuts including weeds. Further it was noted that it is primary pest of soybeans in many parts of the Pakistan. It also cause damage tomatoes due to constant feeding damage to fresh market tomatoes it can reduce crop quality and result in economic loss. The parasitism ratio on the tomatoes was recorded highest (\*49%) and lowest was recorded in the soyabeans (\*\*13.12%) while no parasitism was observed on the grains during present survey.

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**SECTION – I I I****E N T O M O L O G Y****PREVALENCE OF DIFFERENT GENERA OF MOSQUITOES AT TEHSIL  
JAHANIA (DISTRICT: KHANEWAL)**AMJAD FAROOQ<sup>1</sup>, HAFSA MUNIR<sup>1</sup> AND AMIR HAMZA<sup>2</sup><sup>1</sup>*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.*<sup>2</sup>*PARC Multan Research Station*

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Mosquitoes are small insects present in all the regions of the world except Antarctica. They are the vector of many diseases like malaria, yellow fever and dengue etc. Present work is on the prevalence of different genera of mosquitoes in Tehsil Jahania district Khanewal. No published informations are available in the mentioned area. This study is an attempt to provide a comprehensive knowledge about the genus composition and seasonal population density of mosquitoes in said area. Different site are selected for the study purpose to find out the variation of population with respect to habitat. Sampling of resting mosquitoes was carried out with the help of aspirator and insecticide spray for half an hour at dawn time on daily basis from Jan. 2013 to May 2013. The total collection contained 727 mosquitoes out of which 72% were females 28% were males. The collection contained two genera viz; *Culex* (56%) and *Anopheles* (44%). *Culex* was present in all the localities (urban and rural) while *Anopheles* was present in 4 localities of our studies (urban) The populations of mosquitoes fluctuate with change in temperature. Maximum population of *Culex* and *Anopheles* was observed during April and decline with the increase in temperature.

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**DIVERSITY OF MAYFLIES (INSECTA: EPHEMEROPTERA) OF RIVER  
NARMADA, MADHYA PRADESH, INDIA**

IMTIYAZ TALI AND ANIS SIDDIQUI

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Ephemeroptera is an important group of insects used in the bioassessment and monitoring of freshwater bodies worldwide because of their relative abundance in a wide variety of substrates and their increasing chances of detecting pollution impacts. The present investigation was carried out to enumerate the biodiversity of ephemeroptera fauna throughout various selected sampling stations of river Narmada. Five sampling sites viz; Punasa Dam (Narmada Nagar), Omkareshwar, Maheshwar, Khalgat and Koteswar (Barwani) were sampled quantitatively. During present investigation, 17

species comprising of 6 families were recorded including Baetidae, Caenidae, Ephemeridae, Ephemerellidae, Heptageniidae and Leptophtebiidae. The dominant family was Baetidae of which *Baetis simplex* was the most common species. The value of Shannon and Weaver Index was found within the range between 0 and 2.597.

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**PREVALENCE OF MOSQUITO LARVAE IN DADU AND LARKANA  
DISTRICTS OF UPPER SINDH**

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Adult mosquitoes are found in an area where favorable resting places, hosts and breeding places are present, (WHO 1975). The ecological habitat which larvae need varies greatly from one species to another, from permanent water surfaces to temporary puddles. During present study we have surveyed different rural localities of Dadu and Larkana districts and identified different temporary and permanent breeding sites of mosquitoes. Larvae were collected by dipping method from irrigation canals, rice fields, marshy areas with a gentle flow of water, sewage water, pools and water canals which seep out of the ground.

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**REVISION OF GENUS *JUNONIA* HÜBNER (LEPIDOPTERA: NYMPHALIDAE)  
ON THE BASIS OF INTERNAL MALE AND FEMALE GENITALIA FROM  
JAMSHORO, SINDH**

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The genus *Junonia* Hübner (1819) belongs to family Nymphalidae. It comprises about 35 species throughout the world; among these five species have been reported from Pakistan (Robert, 2001). They are commonly called buckeyes, being brightly marked with blue and orange, and possessing prominent ocelli. Both sexes are similar in colour and pattern, but the male has more extensive area of blue and smaller ocelli on the hind wings. During present study, 85 specimens were collected from the different localities of the district Jamshoro from March to September 2013. Identification has been done by the colouration of fore wings, mouth parts, external and internal male and female genitalia and observing paratypes. Out of 85 specimens, four species namely *Junonia orithya*, *Junonia lemonias*, *Junonia almana* and *Junonia lemonia* were identified. These species were compared with their closely allied species.

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**DIVERSITY OF BUTTERFLY FAUNA OF DISTRICT DIR LOWER**

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Butterflies have been regarded as the symbol of beauty and grace and also take part in the key stone ecological process of pollination. The present study was conducted on Diversity of Butterfly fauna of District Dir lower KP, Pakistan. Collection was carried out during March - August 2013. This was the first survey of butterfly fauna of District Dir lower. The specimens were collected with the help of scoop net, sweep net and directly by hands. The collected samples were identified with the help of taxonomic keys and preserved specimens in National Insect Museum at NARC Islamabad. The identified species were preserved by placing them in preservation boxes containing naphthalene balls. The collection of 375 specimens were preserved and identification revealed 24 species belonging to 20 genera and 7 families. The following species were identified with body length of  $2.5\pm0.08$ ,  $2.3\pm0.18$ ,  $1.5\pm0.05$ ,  $1.5\pm0.08$ ,  $1.5\pm0.07$ ,  $1.5\pm0.09$ ,  $2.2\pm0.12$ ,  $1.9\pm0.08$ ,  $1.9\pm0.19$ ,  $1.9\pm0.05$ ,  $1\pm0.07$ ,  $0.9\pm0.07$ ,  $1.5\pm0.08$ ,  $2.2\pm0.18$ ,  $2\pm0.07$ ,  $1.5\pm0.07$ ,  $1.7\pm0.05$ ,  $1.7\pm0.05$ ,  $1.6\pm0.11$ ,  $1.9\pm0.08$ ,  $2.1\pm0.23$ ,  $1.9\pm0.07$ ,  $1.4\pm0.08$ ,  $1.6\pm0.24$  and  $1.8\pm0.10$  cm for *Papilio polyctor*, *Papilio demoleus*, *Junonia almana*, *Pararge schakra*, *Junonia hierta*, *Junonia orythea*, *Argyrius hyperbius*, *Hypolimnys bolina*, *Vanessa cashmiriensis*, *Phalantha phalantha*, *Melitea didyma*, *Lycaena phlaeas*, *Lybithea lipita*, *Danius chrysippus*, *Hipparchia parasitas*, *Lethe rohria*, *Maniola davydovi*, *Pontia daplidice*, *Belenois aurota*, *Pieris brassicae*, *Colias erate*, *Eurema hecabe*, *Colias fieldi* and *Cynthia cardui*, respectively. The Highest population was shown by *Pieris brassicae* followed by *Danius chrysippus* and *Cynthia cardui*. Twelve species were identified belonging to Family Nymphalidae (50%), which shows the highest abundance rate. Butterfly density was the highest at Timergara followed by Adenzai. Butterfly fauna was the highest in May followed by August and lowest in March. It is concluded that pollution free environment of Timergara is more suitable for the survival of butterfly fauna.

**INSECT FAUNA OF BRINJAL (*SOLANUM MELONGENA*) IN HAZARA REGION**

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Brinjal (*Solanum melongena* L.) is one of the commonly used vegetable crops by majority of the people and is popular in many countries i.e, Central, South and South East Asia, some parts of Central America and Africa. Although eggplant is a summer crop, it

is can be grown all the year under suitable irrigated condition therefore it is subjected to attack from nursery stage till harvesting by number of insect pests. A survey insect fauna of Brinjal (*Solanum melongena*) in Hazara region was conducted during May to September 2013. The insect were surveyed and collected every fortnight. Insects from fields were collected during day time, for further study the collected specimens were carried to the National Agriculture Research Centre (NARC). A responsible collection of insect was done using active collection techniques which involves physical picking of the individual from the habitat using fine hair brush, wet finger or forceps for relatively slow moving insects, immature stages or sedentary adults that are not able to fly. For fast flying insect net were used having about 60 cm handle and a bag held open by a hoop of 30 cm diameter. During the present study a total of 18 species of insect were collected from the farmer field. The insect species include predator as well as pest of the crop. The partial results of the survey show *Nezara viridula*, as the most abundant specie of insect followed by *Reptortus pedestris*.

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#### **DIVERSITY ANALYSIS OF INSECTS OF THE THORN FOREST COMMUNITY AT HARAPPA ARCHAEOLOGICAL SITE, PAKISTAN**

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The present study was conducted to compare the insect diversity of old groves and newly rehabilitated stands of thorn forest community at Harappa archaeological site. A total of 2201 insects belonging to 136 insect species under 14 insect orders were collected from old groves while 1130 insects belonging to 76 insect species under 13 insect orders were collected from restored sit. Different diversity parameters *i.e.*, Shannon index, Simpson index, Hill diversity index and Sorenson similarity index were used to explain the insect diversity at both the sites. Results showed that only the insects belonging to orders Coleoptera and Dermaptera were significantly less in mean species number ( $p < 0.05$ ) at the restored site while all other insect orders showed no significant difference in their mean species numbers between old groves and restored site. Diversity of all the insect orders was significantly less ( $p < 0.01$ ) at the restored site except Isoptera. Cluster analysis explained the patterns of insect plant associations. After 12 years of restoration both the sites showed 72% similarity in the insect species composition but it is expected that with the increasing age of the plant community greater habitat and food resources will be available that will enhance the diversity of insects on the restored site.

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**BIOLOGY OF MANGO FRUIT FLY *BACTROCERA DORSALIS* (ORIENTAL FRUIT FLY) (DIPTERA: TIPHRITIDEA IN LABORATORY**

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Fruit Fly *Bactrocera dorsalis* is widely distributed throughout the Asia, mostly tropical and sub-tropical areas. It infested more than 250 host plant of fruit and vegetables. In Pakistan *Bactrocera dorsalis* case serious infestation in Mango, Citreous, guava and Vegetables it causes 5-10% yield loss only in mango. Biology of *Bactrocera dorsalis* was carried out in Entomology laboratory Department of Zoology under control conditions on their natural food stored varieties of mango chousa, Beganpali, sohanra . During present study total three larval instars of *dorsalis* were observed, developmental period of larvae recorded 4-5 days, pupal period recorded 6-7 days while adult survive 4-5 weeks male survive 2-3 and female survive 4 weeks. Female starts egg laying after 2 days of mating. Egg hatched after 2 days.

**GRASSHOPPER (ACRIDOIDEA) OF RANIKOT SINDH**

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Ranikot is the most famous historical place of Sindh. This enchanted fort is located in Laki Mountains of the Lake range on the right side of the mighty River Indus at a distance of about 35 km from the present day town of Sann. The most of the 35-40 km long wall is made up of natural cliffs and barricades of mountainous rocks at places rising as high as 2000 feet sea level. As one enters the fort one can find hills, valleys, streams, ditches, ponds, pools, fossils, building structures, bastions watch towers ravelins ammunition deposits, fortress-all inside ranikot , adding to more its beauty and mystry a spring emerging from an underground water source near the Mohan gate is named as Parryan jo taru ( the spring of fairies). Although considerable work has been carried on the Geology and Plant biodiversity of this fort but no attention has been paid to animal biodiversity. During the present study diversity and distribution of Orthopteroid Insects of Ranikot Fort has been studied for the first time. The collected material was sorted out into : *Dericorys tibialis* (Pallas, 1773) of family Dericorythidae, *Eremopeza gigas* (Kirby)F, *Eremocharis* spp. of Pamphagidae: *Chrotogonus trachypterus trachypterus* (Blanchard), *Chrotogonus trachypterus robertsi* (Kirby), *Pyrgomorpha bispinosa deserti* (B.Bienko) of Pyrgomorphidae while from Family:Acrididae:Subfamily Oxyinae, *Oxyhyla hyla* Audinet-Serville,183, Subfamily Hemiacridinae: *Spathosternum prasiniferum* (Walker, 1871),Subfamily Cyrtacanthacridinae: *Anacridium rubrispinium* B. Bienko,



1948, *Cyrtacanthacris tatarica tatarica* Linnaeus 1758 *Schistocerca gregaria* (Forsk. 1775); Subfamily (Calliptaminae) *Acorypha glaucopsis* (Walker, 1870), *Sphodromerus undulatus undulatus* (Kirby, 1914); Subfamily Eyprepocnemidinae: *Eyprepocnemis alacris impicta* (Uvarov) *Choroedocus illustris* (Walker, 1870), *Heteracris littoralis* (Rambur, 1838), *H. adspersa* (Redtenbacher, 1889). The most significant finding are the species of Pamphagidae at low sea level. Subfamily Oedipodinae: *Acrotylus humbertianus* Saussure, 1884 *Aiolopus thalassinus thalassinus* (Fabricius, 1781.), *A. thalassinus tamulus* (Fabricius, 1798), *Hilethera aeolopoides* (Uvarov, 1922), *Locusta migratoria* (L. 1758). *Sphingonotus savignyi* Saussure 1884. *S. rubescens rubescens* (Walker, 1870), *Trilophidia annulata* (Thunberg, 1815). Subfamily Acridinae: *Acrida exaltata* (Walker, 1859) *Duroniella laticornis* (Krauss, 1909), *Truxalis eximia eximia* (Eichwald, 1830) *T. fitzgeraldi* Dirsh, 1950 Subfamily Gomphocerinae: *Gelastorhinus semipictus* (Walker, 1870) *Gonista rotundata* Uvarov, 1933 *Mesopsis cylindricus* (Kirby, 1914), *Ochrilidia geniculata* (I. Bolivar, 1913) *O. gracilis gracilis* (Krauss, 1902).

#### ESTIMATION OF EXTENT OF DAMAGE OF SELECTED SPECIES OF LEPIDOPTERA ON SELECTED HOST PLANTS

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Lepidoptera is an order of pest of economical importance. Quantification of foraging of four selected species i.e. *Pieris brassicae*, *Spodoptera exigua*, *Helicoverpa armigera* and *Trichoplusia ni* were carried out. Four pre-weighted part of crop plant viz. Brassica, Cabbage, Cauliflower and Turnip were offered as feed. Readings were taken on per day basis. *P. brassicae* was found to be most destructive species as compared to remaining three with maximum overall consumption ( $14.67 \pm 4.566$  g), while *H. armigera* ( $11.765 \pm 4.756$ ), *T. ni* ( $10.707 \pm 4.623$ g) and *S. exigua* ( $8.99 \pm 3.925$  g) *P. brassicae* showed maximum consumption on brassica ( $21.36 \pm 0.72$ g), *T. ni* on Cabbage ( $16.90 \pm 0.59$  g), *H. armigera* and *S. exigua* on Cauliflower ( $18.04 \pm 0.68$  and  $14.01 \pm 0.16$  g, respectively) during whole of their larval duration. Turnip was least preferred diet by all the four species. Maximum per day consumption was calculated for *T. ni*, (1.03g) followed by *P. brassicae* (0.965g) *H. armigera* (0.807g) and *S. exigua* (0.7635g). Similar trend was observed in pupal weight 0.505g, 0.432g, 0.34g and 0.0.268g for *P. brassicae*, *H. armigera*, *T. ni* and *S. exigua* respectively. But feeding index was maximum for *H. armigera* (0.0368) followed by *P. brassicae* (0.0344), *T. ni* (0.0318) and *S. exigua* (0.0297). Data was analyzed by applying analysis of variance followed by tucky contrast and significant differences were observed.

### **DIVERSITY AND DISTRIBUTION OF BUTTERFLIES IN DISTRICT MUZAFFARABAD, AZAD KASHMIR**

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A study was conducted to document the butterfly species diversity and investigate the relationship between climatic variations (rainfall, humidity, temperature) in District Muzaffarabad from May to August, 2012. Butterflies were collected from various localities of District Muzaffarabad viz; Pattika, Kohori, Brarkot, Muzaffarabad City, Garhi Dopata, Domail, Shaheed Gali, Chatterkalas and Kohala. Adult butterflies were collected from fields, gardens, vegetation and forest with the help of a hand net per hour from May to August, 2012 during 11.00 A.M to 3.00 P.M. A sum total of 32 species butterflies belonging to eight families and seventeen genera have been captured and identified. Out of 886 butterflies observed, the highest butterflies abundance (n=139) was recorded in Chatterkalas while lowest abundance (n=37) in Kohori. The influence of environmental factors (Temperature, Humidity and Rainfall), vegetation and altitude on the distribution and abundance of butterflies was recorded. It has been observed that temperature and humidity played important role in distribution of butterflies. Positive correlation was observed between the number of butterflies ( $p=0.010$ ), temperature ( $p=0.000$ ) and humidity ( $p=0.000$ ). However, numbers of butterflies were found to be negatively correlated with altitude. Species richness and abundance were higher in mid altitudes while lower in high and low altitudes. Distribution of butterflies was found to be associated with dominated vegetation like *Ficus palmate*, *Olea fregeneae*, *Canabus sativa*, *Populus ciliate*, *Mytinus royleanus*, *Elymus Canadensis* etc. The comparison of average temperature and humidity of year 1995 and 2012 in different localities of District Muzaffarabad during four months viz; May, June, July and August exhibited significance variation in temperature and humidity between these years. Likewise, significant increases in species richness and species abundance have been recorded in present study compared to previous study in 1995.

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### **EFFECT OF NATURAL AND ARTIFICIAL DIETS ON THE LIFE HISTORY PARAMETERS OF MELON FRUIT FLY *BACTROCERA CUCURBITAE* (COQUILLET)**

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Fruit flies are the noxious pests of fruits and vegetables throughout out the tropical and subtropical regions of the world. The melon fruit fly *Bactrocera cucurbitae* is a

polyphagous pest of vegetables and fruits. We evaluated the effect of natural bottle gourd, (*Lagenaria siceraria*) and artificial diets on the life history parameters of *B. cucurbitae* under lab conditions. Results revealed that shortest incubation period ( $3.0 \pm 0.54$ ) was observed on natural diet whereas; lowest hatching % ( $12.4 \pm 2.11$ ) was observed on blotting papers. However, reduced larval duration ( $5.6 \pm 0.24$ ) was observed when maggots were provided with *bottle gourd* as compared with artificial diet ( $6.6 \pm 0.24$ ). Furthermore, significantly ( $p < 0.05$ ) higher pupal recovery, pupal duration and adult emergence were recorded on natural diet. In contrast, statistically higher pupal weight ( $p < 0.05$ ) was observed on artificial diet. In addition, number of deformed adults was higher in natural diet as compared to artificial diet. These findings could be helpful in defining more optimum conditions for the mass rearing of *B. cucurbitae* for use in Sterile Insect Technique (SIT), programmes for various orchards.

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**REARING OF SAW-TOOTHED GRAIN BEETLE, *ORYZAEPHILUS SURINAMENSIS* (L.) (COLEOPTERA: SALIVANDAE) ON DIFFERENT STORED VARIETIES OF DATE PALM FRUITS OF SINDH, PAKISTAN**

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Date-palm, *Phoenix dactylifera* (L.) is an important cash and commercial crop of Pakistan. In Pakistan, Sindh is mother land for cultivating the different varieties of dates. That is why, the many varieties of dates are stored whole year in huge amount. But stored dates are facing a serious problem of insects infestation especially by Saw-Toothed Grain Beetle, *Oryzaephilus surinamensis*. It has been found in high numbers in stored date palm fruit. However, rearing of this pest is not a simple task, mainly because of its feeding behaviour, small size and high mobility. Thus, the aim of this research was to develop a simple rearing method for *Oryzaephilus surinamensis* using stored date palm fruits. The adult insects were collected from storage infected dates in Khairpur region of Sindh, Pakistan. The 10 pairs of specimen were placed inside the plastic jars with various dates varieties for a period of three months at room temperature. The observations were made weekly. It was observed that one life cycle (egg to adult) completed almost in 45 days. This research was carried out in the Entomology laboratory University of Sindh Jamshoro. Rearing of *Oryzaephilus surinamensis* on various stored date fruit varieties viz; Kupro, Karbalian, Fasli, Aseel and Dadhi for three months 01-02-2013 to 31-04-2013 to observe the growth rate of *Oryzaephilus surinamensis*. The growth rate was observed in five varieties of date fruit viz; Kupro, Karbalian, Fasli, Aseel and Dadhi. The numbers of eggs, larvae, pupae and adults was counted after three months for storing period. In Kupro (eggs 125, larvae 80, pupa 55, adults 450), Karbalian (eggs 105, larvae 55, pupa 45, adults 350), Fasli (eggs 90, larvae 30, pupa 35, adults 250), Aseel (eggs 55, larvae 20, pupa 25, adults 180), Dadhi (eggs 40, larvae 10, pupa 15, adults 130) were

observed. The growth rate was observed the highest in Kupro variety and the lowest in Dadhi variety. The larvae and adults both feed voraciously on dates. The moisture and sugar was more in Kupro and less in Dadhi. The Kupro variety has also soft and tender flesh, so that the beetles were easily stay and feed. The growth rate showed significantly positive correlation with moisture, carbohydrates and soft flesh.

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**SOME STUDIES ON THE EMERGENCE AND PHENOLOGY OF *SCELIO*  
(HYMENOPTERA: SCELIONIDAE) FROM SINDH**

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*Scelio* (wasps) are regarded as important parasitoids of acridids eggs through out the world including Sindh. During the present study the development time of *Scelio* was reported significantly greater than the duration of embryonic development of the host. Adult *Scelio aegypticus* Priesner emerged in 14 days after the hatching of *Hieroglyphus nigrореpletus* Carl under laboratory condition however, in the field, pharate adults with in host eggs and active, newly emerged wasps on egg beds found at the 2<sup>nd</sup> and 3<sup>rd</sup> instars of grasshopper while some *Scelio* emerge after the three week when the upper most eggs in the host egg pod were fully parasitized. It was observed that delayed in the emergence of *S. aegypticus* results in the emerging host nymphs being trapped and dying below the unemerged parasitized eggs. The present study might be helpful for adapting the control measures at more appropriate time.

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**BIOLOGICAL STUDIES ON THE PAINTED GRASSHOPPER *POEKILO CERUS*  
*PICTUS* (F) FROM DISTRICT SHIKARPUR.**

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Studies on the biology of the *Poekilocerus pictus* (F) was carried out under laboratory conditions. There were two successively seasons i-e summer season and autumn season was observed for *P. pictus*. The insect laid eggs in moist soil at depth of 6-8 inches deep ,eggs pods were hard after drying it, Beside this slight variations were noted in the morphology of egg pods and egg's of *P. pictus*. The size of egg pods was calculated ( $45.11 \pm 1.19$  mm) and weight was (0.82 gms). Similarly egg's size was calculated ( $7.75 \pm 0.25$  mm) for fresh egg's and ( $6.07 \pm 0.61$  mm) for dry eggs. *P. pictus* laid maximum ( $1.8 \pm 0.78$ ) egg pods during entire period of life and total no of eggs per pod were counted ( $79.8 \pm 16.12$ ) .The total nymphal period ranges from ( $62.60 \pm 5.98$  days)

with six stages range. The pre-oviposition and oviposition period were observed ( $14.5 \pm 1.43$  days) and ( $2.28 \pm 0.66$  days) respectively. Adult longevity of male was recorded ( $79.4 \pm 4.15$  days) while in female ( $80.75 \pm 9.11$  days). Total mating before egg laying was noted ( $14.3 \pm 2.1$ ). Present contribution will provide brick to knowledge how to adopt control measure at appropriate time.

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**PRELIMINARY STUDY ON THE LONG HORNED MONKEY HOPPER  
(EUMASTACIDAE: GOMPHOMASTICIDINAE) FROM DISTRICT  
ABBOTABAD, KHYBER PAKHTUNKHWA**

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At the present 103 specimen of monkey hopper were collected from district Abbotabad Thandiani. The collected material was sort out in to single genus *Gomphomastax* (Brunner Wattenwyl 1898) with few species presently only one species namely: *G. antennata* (Brunner Ven. Wattenwyl 1998) has been identified while remaining are in the process of identification. During the present study it was observed that these were small to medium sized wingless insects mostly distributed in high mountain of Abbotabad valley. The species of this specimen were collected from the herbaceous flora at the height of 3500-4000 m high Mountain. This work is an initiative step toward the identification of the Eumastacidae from the region.

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**STATUS OF AWARENESS OF INSECT POLLINATION IN SOME SELECTED  
AREAS OF PAKISTAN**

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A survey for awareness of pollination was conducted during 2011 in Study, Education, Evaluation, Promotion (STEP) sites located at Murree and Multan Thirty farmers at each site were interviewed. In addition agriculturists, scientists, extensionists, lecturers, students, media persons, NGOs were also interviewed 10 each. Results of farmers in STEP sites showed the fact that many farmers lump pollinators together with insect pests. The analyzed data revealed that 53% farmers do not have knowledge about pollinations. The farmers at STEP site Multan were more aware than farmers at STEP site Murree. Of the knowledgeable farmers about 70% believe that pollination do not affect crop yield. More than 50 % farmers do not have idea that what are necessary for pollination to take place. About 40% believe that air, insects and bees are necessary for

pollination. Majority (86%) responded that human being is doing nothing for pollination to take place. About 68% farmers are of opinion that they leave insects alone because they have no interest in it. Furthermore 25% farmers said that these should be killed through spray as they damage their fruits. Similarly 45% farmers consider that pollinators are important and suggested that pollinators should be protected from pesticide spray. All farmers (100%) felt that the various Government departments had not been proactive in promoting the awareness of pollination and the need to protect them. No organization promoted any direct conservation needs and/or practices directly related to pollinators. Other groups agreed that pollination is important for crop production except 2 % journalists. All consider insects, air and bird are necessary for pollination to take place. All the target groups agreed that yield increases.

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**A NEW SPECIES OF *SCHIZODACTYLUS* (SCHIZODOCYLINAЕ:  
SCHIZODACYTIDAE) FROM SINDH, PAKISTAN**

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A new species of genus *Schizodactylus* namely *S. sindhensis* described from river Indus. This new species is large in size than all known species in the world. Furthermore this new species is differs from all other species of *Schizodactylus* having large size and the hind tibia posses four spines on the inner and outer side.

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**STUDY ON VARIATION IN OVIPOSITOR OF PHANEROPTERINAE  
(ORTHOPTERA: TETTIGONIOIDEA) FROM PAKISTAN**

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The Phaneropterinae are of considerable economic importance to agricultural crops, fruits orchards, grapevine, berry shrubs and forests in the world including Pakistan as there is no published record available on the variation in ovipositor of Phaneropterinae from Pakistan. Therefore an attempt has been made to study the variation in ovipositor of Phaneropterinae. At the present significant variation have been observed in ovipositor of *Phaneroptera* species it was observed shorter and laterally compressed and found bent sharply upward. However, it was found strongly flattened with fine serration on the entire upper margin in *Trigonocorypha* on contrary to this it was observed narrow and semi-circularly upward in *Ducetia* species while it was wider in *Holochlora* species. Besides

this in *Tylopsis* species ovipositor was lamelliform and curved from the base. This study might be basic tool for the correct identification of species. This study was financially supported by Pakistan Science Foundation Islamabad under Research Project No PSF/S-SU/BIO (423).

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**CLADISTIC ANALYSIS OF THE GENERA OF FAMILY PHILOPTERIDAE  
(PHTHIRAPTERA: ISCHNOCERA), FROM KARACHI, PAKISTAN**

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The phylogenetic relationship of eleven genera of the family Philopteridae (Phthiraptera: Ischnocera) were studied from Karachi region, Pakistan. These genera were analyzed cladistically, using their apomorphic characters. A generic key of family Philopteridae has also been formulated and cladogram is presented and discussed.

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**STUDIES ON THE GENUS *EUCONOCEPHALUS* KARNY, 1907  
(ORTHOPTERA: TETTIGONIOIDEA: CONOCEPHALINAE) FROM  
PAKISTAN**

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The grasshopper insects belonging to the genus *Euconocephalus* are of considerable economic importance in Pakistan. They cause constant threat to the valuable crops in Pakistan and cause economic loss to mankind. They are commonly known as the Cone-headed grasshoppers. During the present survey a total of 851 specimens of Conocephalinae pertaining to genus *Euconocephalus* were collected from various localities of Pakistan the material was sorted out into 06 species *i.e.* *Euconocephalus incertus*, Walker 1869 *Euconocephalus indicus* (Redtenbacher, 1891), *Euconocephalus nasutus* Thunberg, 1815, *Euconocephalus mucro* de Haan, 1842 and *Euconocephalus pallidus*, Redtenbacher, 1891, and *Euconocephalus* sp. Beside this, morphological characters, measurement of body parts and distribution of genus has been provided from Pakistan. This study was supported by grants received from Pakistan Science Foundation Islamabad for Research Project No PSF/S-SU/BIO (423).

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**SYSTEMATIC STUDY OF OXYINAE (ORTHOPTERA: ACRIDIDAE) FROM SUKKUR DIVISION, SINDH, PAKISTAN**

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At the present an extensive survey for the collection of grasshoppers was carried out during the year 2012-2013 from various localities of Sukkur division. A single genus with four species *i.e.*, *Oxya velox* (Fabricus), 1787, *Oxya hyla hyla* (Serville), 1831, *Oxya fuscovittata* (Marschall), 1836, *Oxya bidentata* (Willemse), 1925, were recorded in large numbers. In addition to these important taxonomic characters along with drawing lines, and important host-plants were also studied. The data obtained from this study might be proved beneficial for planning agencies in near future.

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**STUDIES ON THE NATURAL LEVEL OF FUNGAL INFECTION IN GRASSHOPPER COLONIES IN UPPER SINDH**

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The infection of grasshopper by naturally occurring entomopathogenic fungi *i.e.* *Aspergillus niger* Van Tieghem (1867), *A. flavus* Jahan Heinrich Friedrich Link (1809) and *A. fumigatus* Fresenius (1863) was noted at the three sites viz: 1<sup>st</sup> site consist on (Khairpur, Sukkur and Ghotki), 2<sup>nd</sup> site (Kashmore and Jacobabad) and 3<sup>rd</sup> sites (Shikarpur and Larkana). Significantly large numbers of grasshopper were collected between the Aprils to Octobers 2013 from various agricultural fields. Mostly the collected species barring the first instars, then these were incubated in rearing cages length 16.5cms, width 13.5cms under laboratory (25°-23'N, 68°-24'E) condition where the temperature fluctuated between 28±2°C to 39±2°C and humidity was 26% to 61 %. From the 1<sup>st</sup> site 873 individual of 07 grasshopper species were incubated, while on the 2<sup>nd</sup> site 432 individual of 10 grasshopper species were incubated. Similarly on the 3<sup>rd</sup> site 723 specimen comprising on 13 species of grasshopper were incubated. At the present it was observed that three studied entomopathogenic fungi were found infecting grasshoppers. A significant difference was noted in the time to death of small grasshopper individual infected with *Aspergillus niger* compared to larger individual. Furthermore, as a result of the examination of dead samples of grasshopper, fungal contamination was found significantly higher *i.e.* -43% in 1<sup>st</sup> site followed by 36% in 3<sup>rd</sup> site and 21 % in 2<sup>nd</sup> site.

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**PRELIMINARY OBSERVATION ON THE IMMATURE STAGES OF  
EYPREPOCNEMIDINAE (ACRIDIDAE: ORTHOPTERA) FROM SINDH**

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Grasshoppers are the potential pest of all plant trees and crops. They destroy all vegetation within a region more completely than any other insect pest. Amongst these grasshoppers species Eyprepocnemidinae causes serious damage to valuable crops. During the field survey it was observed that immature stages of grasshoppers are more injurious to crops due to lacking of wings. It was therefore, felt necessary and an attempt has been made to study the life-cycle of this pest. Actually, species of Eyprepocnemidinae comprises on six nymphal instars and all the developmental stages having significant difference with each other. Furthermore, Identification key and measurement of different body parts are also provided for easily reorganization of instars.

**FOUR NEW RECORDS OF CRICKETS (GRYLLIDAE: ORTHOPTERA) FROM  
HYDERABAD AND ITS ADJOINING AREAS, SINDH, PAKISTAN**

S. MALIK, T.J. URSANI, N.M. SOOMRO AND NEELAM ANSARI  
*Department of Zoology, University of Sindh, jamshoro*

Fifteen species of crickets were identified out of which four namely, *Acheta thoracicia* Saeed *et al.* *Turanogryllus bullahi* Saeed, *Phonarellus minor* and *Modicogryllus baroculus* Saeed are the new records first time reported from this area.

**ON THE INCIDENCES OF OEDIPODINAE (ORTHOPTERA: ACRIDIDAE)  
FROM NARA DESERT KHAIRPUR, SINDH, PAKISTAN**

FAKHRA SOOMRO, MUHAMMAD SAEED WAGAN AND RIFFAT SULTANA  
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A detailed survey for the collection of short horned grasshopper species was carried out during the year 2012-2013 from different localities of Nara desert *i.e.* Choondiko, Khehwari, Tajal, Sikandarabad and Jubo. A total of 322 specimens were collected and sorted into 6 genera and 8 species viz., *Sphingonotus rubescens rubescens* (Walker, 1870), *S. savignyi* (Saussure, 1884), *S. theodori theodori* (Uvarov, 1924), *Aiolopus thalassinus thalassinus* (Fabricius, 1781), *Hilethera aeolopoides* (Uvarov, 1923), *Acrotylus humbertinus* (Saussure, 1884), *A. longipes subfasciatus* (Bei-Bienko,

1948), *Trilophidia annulata*(Thunberg, 1815), *Locusta migratoria* (Linnaeus, 1758), of wing band grasshoppers (Oedipodinae). Furthermore the status some species were also conformed from this area. Hopefully present study might be proved as base line data for future research.

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**PRELIMINARY STUDY ON MORPHOLOGY AND PHALLIC COMPLEX OF  
GENUS *OEDALEUS* FIEBER (ACRIDIDAE, ACRIDOIDEA, ORTHOPTERA)  
FROM PAKISTAN**

BARKAT ALI BUGHIO, RIFFAT SULTANA, MUHAMMAD SAEED WAGAN AND  
SABIR ALI CHANNA.

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Grasshoppers fauna in Pakistan are known as richest one because of more than 80% agricultural fields; vegetations herbs and shrubs. To know about orthopterans the author were studies three species of the genus, they are *Oedaleus abruptus*, Thunberg; *O. roscens* Uvarov, and *O. senegalensis* Krauss. The main component of diagnosis to studies external morphology, their incidence, phallic complex and distribution throughout Pakistan. When their numbers increased they pose threat to fodder crops by eating the cropping portion of plants. They are seen enormous during March to end of October. These three species are called as fascinated from coloration point of view and can be categorized as graminivorous. Epiphallic characters are considered authentic to diagnose accurately. The distribution of these three species at province level are also considered.

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**PRELIMINARY STUDIES ON THE INFECTION OF STORED GRAIN MITES  
FROM SINDH**

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Mites are considered important pest of stored products all over the world including Sindh. At present infection of 07 species belonging to 03 genera *i.e.*: *Capronomia*, *Histiosoma* and *Glyphanoetus* were recorded on the wheat, rice, maize and other stored products available in store houses. Further, it was also observed that mites are also responsible for the dissemination of various pathogens fungi in stored food and seeds. Moreover, significant co-relation of maximum temperature and mite population was also noted. Present study might be useful as special approach to problem of control and a timely organization of a wide variety of methods.

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**FAUNASTIC STATUS OF GENUS *NEOHALYS* AHMED AND PARVEEN  
(HEMIPTERA, PENTATOMIDAE, HALYINI) OF PAKISTAN**

ABDUL MANAN SHAIKH<sup>1</sup>, NASREEN MEMON<sup>2</sup>, NADIR ALI BIRMANI<sup>1</sup> AND  
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During present studies specimens of the tribe Halyini were collected from different provinces of Pakistan and identified with keys and available literature. Genus *Neohalys* Ahmad and Parveen (1982) is recorded from different location of Punjab and NWFP, Pakistan with all three known species. This new genus has been compared with two genera *Halys* Fabricius and *Salixocoris* Ahmad and Abbasi.

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**COMPARATIVE EFFICACY OF ORDINARY SOAP, SIMPLE SHAMPOO AND  
ANTI-LICE SHAMPOO AGAINST HEAD LOUSE, *PEDICULUS CAPITIS*  
(PTHTHIRAPTERA: PEDICULIDAE).**

NIDA IDRESS, QAMAR SAEED\*, SHAFQAT SAEED, UMAIR SIAL AND  
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*Pediculus capitis* (Pththiraptera: pediculidae) or head louse is the contagious pest of human which can be spread by sharing personal belongings or closed contact. This research encompasses the treatment of human head (generally of diameter of 15 inches) by ordinary soap, simple shampoo and finally three anti-lice shampoos for the control of this pest. For ordinary soap and simple shampoo, the number of nits, nymphs and adults were counted up to three washings. Washing with ordinary soap reduced the number of nits, nymphs and adults significantly up to third washing. But the number of nits, nymphs and adults reduced significantly up to second or third washing by simple shampoo. Three anti-lice shampoos were treated naming Medicam, English and Serne up to two washings. Medicam shampoo did not show significant reduction in number of nits, nymphs and adults while English anti-lice shampoo reduced the number of nits, nymphs and adults significantly after second washing.

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### COMPARATIVE STUDY ON THE ANATOMY OF GRASSHOPPER INFECTED WITH *MERMIS NIGRESCENS* FROM SINDH

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At the present a total of 403 specimen of grasshopper comprising on 7 species were collected from different climatic zone of Sindh. Out of 403 specimens 267 were infected with *Mermis nigrescens* were found severely infected while 136 were found uninfected. It was observed that major modifications of the hosts metabolism by *M. nigrescens* parasitism was manifested in host tissue degeneration and retarded development including resorption or suppression of oocyte development. Furthermore, it was also noted that hosts were usually prevented from maturing to the adult stage or when they do, they were unable to reproduce. Besides this, Parasitized males showed very little difference in the development of the testes when compared to normal males and similar observation was also reported for female.

### A REVISION OF ASIAN SPECIES OF TRIBE HALYINI (PENTATOMIDAE: PENTATOMINI)

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The tribe halyini Stal is a polyphagous, heterogeneous group of stink bugs, which has a worldwide distribution; found in Palearctic and oriental regions of America, Australia, Europe and Asia. Present study includes the revision of thirty one Asian genera; *Agaeus* Dallas 1851, *Asyla* Walker 1867, *Ameridarpa* Ghauri 1982, *Apodiphus* Spinola 1837, *Cahara* Ghauri 1887, *Carenoplistus* Jakovlev 1882, *Dalpada* Amyot & Serville 1843, *Dendrites* Kirkaldy 1909, *Erthesina* Spinola 1837, *Eupaleopada* Ghauri 1982, *Halys* Fabricius 1803, *Iskenderia* Kiritschenko 1963, *Izharocoris* Afzal & Ahmed 1981, *Jugalpada* Ghauri 1975, *Lodosocoris* Ahmed & Afzal 1986, *Meridarpa* Ghauri 1982, *Meridindia* Ghauri 1982, *Mustha* Amyot & Serville 1843, *Nevisanus* Distant 1893, *Neohalys* Ahmed & Parveen 1982, *Neolodosocoris* Memon & Ahmed 2002, *Neonevisanus* Distant 1918, *Orthoschizops* Spinola 1852, *Paranevisanus* Distant 1908, *Phricodus* Spinola 1839, *Salixocoris* Ahmed & Abbasi 1974, *Saontarana* Distant 1918, *Sarju* Ghauri 1977, *Surenus* Distant 1901, *Tachengia* China 1925, and *Tipulparra* Ghauri 1980. These species are polyphagous. These genera are revised on the basis of their external morphology (shape and length of paraclypei, number of antennal segments, shape of lateral margins, and humeral angles of pronotum) and external and internal male (shape of paramere) and female genitalia (shape and number spermathecal bulb processes).

**STUDIES ON THE IMMATURE STAGES OF ACRIDINAE (ORTHOPTERA: ACRIDIDAE) FROM SUKKUR DIVISION**

SAJJAD ALI LARIK,<sup>1\*</sup> RIFFAT SULTANA,<sup>1</sup> MUHAMMAD SAEED WAGAN<sup>1</sup> AND IMRAN KHATRI<sup>2</sup>

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Grasshoppers belonging to Acridinae are Polyphagus insects and cause severe damage to agricultural crops. Of these *Acrida exaltata*, *Acrida gigantea* and *Truxalis examia examia*, were reported as severe pests of cash crops in Sindh particularly Sukkur division. During present study it was noted that immature stages of these species were more injurious to the crops. It was therefore felt necessary and attempt has been made to study the biology of these pests. Moreover, Identification key and measurement of different body parts were also taken for easily identification of instars. The present study might be helpful for taking possible control measure against the economic species may be initiated at early stage.

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**STUDY ON THE GENUS *KUKULCANIA* LEHTINEN, 1967 (ARANAE: FILISTATIDAE) FROM PAKISTAN**

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The genus is one of the most abundant members and distributed in the Southern America. It is the sedentary and hidden in crevices walls, rocks, foliage and house spider. During the present study single genus has been described with single species namely *Kukulcania hibernalis* (Hinds, 1842) reported as beneficial predator feeds on termites. It is interesting to note that this genus has been reported for the first time from Pakistan.

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**INSECT POLLINATOR FAUNA OF APRICOT FROM GILGIT-BALTISTAN**

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National Insect Museum, NARC, Islamabad

Surveys were made for apricot insect pollinators from Gilgit-Baltistan during blossom in 2010 and 2011. Thirty two (32) species of 16 genera under 8 families of orders Diptera, Hymenoptera and Lepidoptera were identified. Order Diptera was represented by family Syrphidae with four species. Five (5) families of order Hymenoptera were recorded *i.e.* Megachilidae with two (2) species, Apidae with 12

species, Halictidae with four (4) species, Andrenidae with five (5) species and Vespidae with three (3) species. While order Lepidoptera was represented by two (2) families, Nymphalidae and Papilionidae with single species each. Among explored fauna, dipterous genus *Scaeva* with its species *Scaeva selenitica*, hymenopterous genus *Lassioglossum* and the *Xylocopa acutipennis* were new records for Pakistan.

### **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 them 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.

### **PREVALENCE AND TAXONOMIC STUDY OF THREE SPECIES OF GENUS *EUPEODES* (DIPTERA:SYRPHINAE) FROM DIFFERENT LOCALITIES HYDERABAD SINDH, PAKISTAN**

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The genus *eupeodes* (Osten Sacken, 1877) is one of the biggest genera of family (Syrphidae:Syrphinae). This genus has oval abdomen and different pattern of stripes present on tergites. Present study was conducted on hoverflies from Hyderabad, Sindh. The specimens were collected during the period of November 2012 to October 2013. We surveyed different localities of Hyderabad division ( Matiari, Tando Jam and Jamshoro). About 650 specimens were collected from different host plants like wheat, brassica, vegetable (tomato, chilli) and weeds. Out of these 300 were males and 250 were females. Three species of genus *Eupeodes*, *E. corolla*, *E. luniger* and *E. latifasciatus* were identified. The larvae of all these three species are aphidophagous and voraciously feed on aphids. Identification was carried with the help of pattern of spots, lunules or bands on the upper side of abdomen and verified by literature. Population of genus *Eupeodes* starts to increase from January to April and decline in the month of May to September, highest number was recorded during the month of February. It is a new record of these three species surveyed from different localities of Hyderabad, Sindh, Pakistan.

**SYSTEMATIC AND ABUNDANCE OF GROUND BEETLES (CARABIDAE: COLEOPTERA) FROM DISTRICT POONCH AZAD KASHMIR, PAKISTAN**

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Abstract: Present study was conducted during 2010- 2012 dealing with the exploration of carabid fauna and study of their systematic from district Poonch of Azad Kashmir, Pakistan. Carabid beetles were collected with the help of pitfall traps and identified up to specie level with the help of available literature. We identified five species under three genera belonging to 3 sub-families. These sub families are Licininae, Carabinae, Brachininae and the species are *Carabus caschmirensis*, *Chlaenius quadricolar*, *Pheropsophus sobrinus*, *Chlaenius laticollis*, and *Chlaenius hamifer*. *Carabus caschmirensis* was the most abundant species. It was followed by *Chlaenius quadricolar*, *Pheropsophus sobrinus*, *Chlaenius laticollis*, and *Chlenius hamifer*.

**SYSTEMATIC STUDY OF THE GENUS *ATTRACTOMORPHA*, SAUSSURE, 1862 (ORTHOPTERA: ACRIDOIDEA: PYRGOMORPHIDAE) FROM UPPER SINDH**

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At the present 02 species of genus *Attractomorpha* Saussure, 1862 i.e. *Attractomorpha crenulata* (Fabricius, 1793) and *Attractomorpha acutipenis* (G. Meneville, 1844) were collected from the agricultural fields from the various localities of the upper Sindh during the year 2013. The collected specimens have been preserved by standard entomological method. In addition to this detail study on taxonomic characters and measurements of body parts is also provided. Hopefully information about this species enhances the Pyrgomorphids wealth of upper Sindh.

**STUDY OF TRIBE ERISTILINI (DIPTERA: MILESIINAE) FROM JAMSHORO, SINDH, PAKISTAN**

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The tribe Eristilini includes hoverflies (Diptera: Milesiinae). Hoverflies are cosmopolitan in distribution and present in all physical environment. Present study was conducted on hoverflies from Jamshoro, the flies were collected through locally

fabricated light trap containing 150 watt mercury bulb fixed at the garden of National Center of Excellence in Analytical Chemistry University of Sindh Jamshoro. collection was done from January to April (2013). Total 300 specimens of species of tribe Eristilini were collected out of which 210 were males and 90 were females. Identification has been done by the wing venation and by observing paratypes.

### **REARING, MARKETING AND DISTRIBUTION OF FIREFLIES FROM SINDH**

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Pakistan insect fauna is very rich, but we are not taking any direct economic benefit from it. Our fancy insects are already traded in the international pet markets. This study is an effort to generate foreign exchange by export of fancy insect's *i.e.* beetle, butter flies and fireflies. In particular attempts were made on fireflies to rear and market it locally. The seasonal distribution and quantum of fireflies in Sind is documented. Fireflies' collection nets are designed for collection, soon after the sunset. They are very much similar like aquarium fishing nets, but the length of linked rode is increase to 10 feet. The authors are rearing fireflies, for stocking in a room of size 8 x 8 x 12 feet since three years, during its season. Humidity in room was increased by using water fountain and "Gule abbasi" Plant, what was collected from the habitat of fireflies. The local price of one adult fireflies is set Rs.100/=. Alhamdo-Lillah it is fixed since 10 years, in Pakistan. The common buyers are Plant nurseries, upper class house holds, etc. it is not distributed in Karachi and its liked rural area. Two species of fireflies are largely distributed in Sindh province at the places namely, Gharo Jam Branch of Babra – Begar Mori, Thatta – Ghoski, Badin Distric – Ghora Bari – Ver - Gail mori, canal – Tando Jam, Agriculture University area, Larkana Rais Canal – Sukkur' rural area, etc.

### **NEW RECORD OF SPECIES OF FAMILY COCCINELLIDAE (ORDER: COLEOPTERA) FROM DISTRICT GHOTKI, SINDH**

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The Coccinellidae are a family of small beetles. It comprises of 5000 species recorded throughout the world. They are commonly yellow, orange, or scarlet with small



black spots on their wing covers, with black legs, heads and antennae. The Coccinellidae are generally considered as useful insects, because many species feed on aphids or scale insects, which are pests in gardens, agricultural fields and orchards. Within the colonies of such plant-eating pests, they will lay hundreds of eggs, and when these hatch the larvae will commence feeding immediately. In the Pakistan very little work has done on the beetles especially from Sindh. Presently we surveyed different localities of Ghotki district of Sindh such as Mirpur, Daharki, Adilpur, Qadirpur etc. from April 2013 to August 2013. 115 specimens were collected out of these four species namely *Psyllobora viginitiduopunctata*, *Cheilomenes sexmaculata*, *Coccinella septempunctata* and *Coccinella undecimpunctata* were identified by observing external and internal male and female genitalia. This is new record of beetle species from the District Ghotki.

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**REVISION AND NEW RECORD OF GENUS *ERISTALIS*  
(DIPTERA:SYRPHIDAE) FROM BHAG NARI, BALOCHISTAN, PAKISTAN**

JUMA KHAN TURK, NASREEN MEMON, NADIR ALI SHAH, DILDAR ALI  
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Syrphid flies commonly known as hover flies and flower flies, they belong to the family syrphidae. Hover flies belong to the *Eristalis* important pollinator and act as scavengers. No noticeable work has been done in Pakistan on this beneficial insect fauna. Two species have been identified: *Eristalis tenax* Linnaeus and *Eristalis arbustorum* Linnaeus under Sub-family Eristalinae. These species have been recorded for first time in Balochistan, Pakistan.

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**SECTION – IV****P A R A S I T O L O G Y****SEASONAL VARIATION OF GULFAM (*CYPRINUS CARPIO*) ON THE  
ECTOPARASITES FROM CARP FISH HATCHERY DISTRICT BADIN, SINDH,  
PAKISTAN**ALI MARDAN JAKHRANI,<sup>1</sup> AZRA ANJUM<sup>1</sup> AND N .T. NAREJO<sup>2</sup><sup>1</sup>*Department of Zoology, University of Sindh Jamshoro*<sup>2</sup>*Fresh water Biology and Fisheries, University of Sindh, Jamshoro*

The study has been carried out period of one year from August 2011 to July 2012 to find out the parasitization of different fish species cultured at carp fish hatchery district Badin with special reference to Gulfam (*Cyprinus carpio*) carp fish. Among 263 specimens of fish examined for parasite. The present study observed the prevalence and intensity in Gulfam (*Cyprinus carpio*) was highest number 81 in the collection followed by Shakur (*Channa punctatus*) 18 and Rohu (*Labeo rohita*) 39 used for the examined of parasites. 17.9% of the total sample was found to be infected by parasites while the mean intensity of the parasites showed a value of 3.8 parasites/fish. It was concluded that the highest infection rate (66.6%) was observed in Gulfam (*Cyprinus carpio*) followed by Shakur (*Channa punctatus*) 50% and the lowest (11.7%) was recorded from Morakhi (*Cirrhinus marigala*) during the present investigation. The prevalence in fish was the highest in the late summer (September to October) and the lowest prevalence was found in the early summer (April to May)

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**PREVALENCE OF SCHISTOSOMIASIS IN BUFFALOES OF BAHAWALPUR,  
PUNJAB, PAKISTAN**MARIA ASGHAR\*, NUZHAT SIAL, ABUL HASANAT, MUHAMMAD SHAFIQ  
CHAUDHARY AND MUAZZAM ALI*Department of Life Sciences, The Islamia University of Bahawalpur*Correspondence: [ahzoologist95@gmail.com](mailto:ahzoologist95@gmail.com)

Schistosomiasis is a snail-born trematode infection of domestic, wild animals and man, and it is worldwide in distribution. During the present study fecal samples from buffaloes were collected on random basis from the all tehsils of Bahawalpur district from February 2012 to October 2012. Of total 1800 fecal samples, 248 (13.8%) were found to be positive. Highest prevalence was recorded in Yazman (18.9%) followed by Bahawalpur (14.4%), Khairpur (13.3%), Hasilpur (12.2%) and the lowest was recorded in Ahmedpur (10%). Monthly overall highest prevalence was recorded in September (29%), while the lowest was found in the month of May (2.5%). Statically chi-square showed a

significant ( $p < 0.05$ ) difference between all areas and months. Overall highest seasonal wise prevalence was found in autumn (26.5%) which was highly significant ( $p < 0.05$ ), followed by a non significant ( $p > 0.05$ ) difference in winter (16%), summer (10%) as compare to spring (7.5%). In overall age wise prevalence the adult buffaloes were found to be highly (16.5%) susceptible than young ones (7%). Overall gender wise prevalence was higher in females (14.8%) than males (10%). Statistically a significant difference ( $p < 0.05$ ) was found between all age and gender groups.

**HISTOPATHOLOGICAL INVESTIGATION OF INTESTINE OF CHICKEN  
(*GALLUS DOMESTICUS*) ASSOCIATED WITH *CHOANOTENIA*  
*INFUNDIBULUM***

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During present investigation desi chickens (*Gallus domesticus*) were brought from different villages of district Hyderabad. Chickens were dissected and their intestine were carefully removed and examined for helminth parasites. The parasites collected from the intestine were preserved, stained and mounted. Tissues from infected intestine were fixed in Bouin's fluid for 24 hours using routine histological techniques, 6-8 micron thick sections were prepared and stained with hematoxylin and eosin finally mounted in Canada balsam. Out of 89 chickens 79 (88.76%) were infected with *choanotenia infundibulum*. Histopathological observations revealed sever intestinal tissue damage which included disintegration, distortion, fusion, inflammation and destruction of architecture of muscular layers, villi and glands.

***ECHINOSTOMA* GHAZII SP.N (TREMATODA: ECHINOSTOMATIDAE  
POCHE, 1962) FROM *EGRETTA GARZETTA* OF SINDH PAKISTAN**

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*Echinostoma* Rudolphi, 1809 Syn: *Echinostominae* Looss, 1899 is a well established genus with numerous species reported from all over the world from Avian hosts. Present species is a first report from District: Jamshoro, Sindh, Pakistan. Ten *Egretta garzetta* were purchased at random intervals. The birds were autopsied in the

laboratory for collection of internal Helminth parasites. Three out of ten birds were found infected with four trematodes. The worms were mounted permanently according to the standard procedure. A detail study was conducted and a new species *Echinostoma ghazii* sp.n is proposed belonging to the genus *Echinostoma* Rudolphi, 1809 from the small intestine of *Egretta garzetta*, Jamshoro, Sindh Pakistan. The new species is characterized by: body is elongated, oral sucker is subterminal, collar spines are smaller in size and 41 numbers, esophagus is comparatively longer, genital opening is post bifurcal, cirrus sac is smaller and terminal uterus joints to form genital opening, acetabulum is larger in size, vitelline glands start from near acetabulum, testes are tandem, irregular lobed, ovary is pretesticular larger than testes situated smaller distance above the testes. Uterine follicles moderate in number and excretory vesicles Y shaped, eggs are oval to elongate in shape and smaller in size.

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**STUDIES OF *EPISTHMIUM* SP. (TREMATODA: ECHINOSTOMATIDAE) IN  
LITTLE EGRET *EGRETTA GARZETTA* IN SINDH, PAKISTAN**

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Six birds little egret *Egretta garzetta* caught from the District, Jamshoro, Sindh, Pakistan. The birds were anesthetized/ autopsied and examined for helminth parasitic infections. Two out of six were found infected with four trematodes, identified as belonging to genus *Episthmium* Luhe, 1909 were recovered from the small intestine of the hosts. The specimens were fixed, dehydrated and mounted, according to standard procedures. The specimens were studied in detail; these are characterized by a Body elongate, small to median-sized. Head collar is reniform, with a dorsally interrupted row of spines. Oral sucker is subterminal and rounded, Pharynx is small than oral and ventral sucker. Acetabulum in anterior half of body. Testes are rounded or somewhat indented, usually large in relation to the body size, tandem, Cirrus pouch anterior to acetabulum. Ovary is rounded, sub median, Uterus is short, with relatively few eggs. Eggs are oval in shape. Vitellaria extending in lateral fields as far forward as pharynx, esophagus or intestinal bifurcation, usually confluent across median line anterior to genital pore and posterior to testes. Parasitic in intestine of birds.

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### **PARASITOLOGICAL AND CLINICAL PARAMETERS OF COCCIDIOSIS IN RABBITS**

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Rabbit coccidiosis is known to be a serious problem; it may be hepatic coccidiosis or intestinal coccidiosis because *Eimeria* spp. inhabits the liver and intestine. Mortality due to coccidiosis causes huge economic loss. The current research was aimed to investigate the prevalence of coccidiosis infection according to temperature, humidity, age and sex variations. A total of 112 rabbits were purchased from 12 different locations of Lahore. Faecal examination was performed carefully by different techniques *i.e.*, direct smear, flotation etc. and the oocyst of *Eimeria* spp. were observed abundantly in rabbits' faecal samples. It was observed that females were slightly more susceptible (47.2%) than males (44.7%); also the incidence of coccidiosis was more pronouncing in baby kits from 1-3 months (25.3%) as compared to adults (15.2%). According to the month wise analysis the infection was more prevalent in month of July, June and March due to high (73.9%, 60.7% & 58.3%) humidity. Coccidiosis also caused changes in blood parameters like increases WBCs (20.8%), neutrophils (17.5%), monocytes (29.1%) and basophils (11.3%) while decreases RBCs (44.9%), Lymphocytes (18.3%) and Hemoglobin level (35.4%). However, eosinophils did not show any change in their percentage.

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### **COMPARATIVE EFFICACY OF TWO BROAD SPECTRUM ANTHELMINTICS AND MODE OF TREATMENT AGAINST INTESTINAL HELMINTHIASIS OF BACKYARD POULTRY**

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Backyard poultry has much importance in poor farming community but its production is not comparable to modern poultry farming owing to repeated exposure to gastrointestinal parasitism. Control of this hidden threat is generally carried out by synthetic dewormers but their treatment frequency is too low when compared with small ruminants. In present trial, sixty naturally infected indigenous poultry birds (2 months age) were purchased from local poultry farm. Selected birds were randomly divided into three groups (20 in each). Two groups were dewormed by Vermisole and Albendazole respectively while third was kept as control under similar management. Moreover, treated groups were further divided (10 in each) based on mode of treatment *i.e.*, mixing of drug

in water and feed. It was observed that both the experimental drugs did not show any considerable reduction in mean eggs per gram (EPG) of feces of gastrointestinal helminthes, however, water mode of treatment showed relatively better results at the recommended dose of manufacturers at day 2 post-treatment (PT). In order to make birds worm free, infected birds were treated again four times at different intervals (day 5, 12, 25 and 40 days) PT to compare changes in different physiological parameters (live weight, blood chemistry, clinical and behavioral changes) between treated and control groups. Significant differences ( $P < 0.05$ ) in EPG of treated and worm free were observed at all treatment intervals. Decrease in EPG of treated group was not in order and it was lowest at day 12 PT and then gradually increased at days 25 and 40 PT. Water mode of treatment showed relatively better results than that of feed, however, both mode of treatment were inefficient that might be due to poor efficacy, low dose recommendation or owing to development of anthelmintic resistance in prevalent helminth species.

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#### EPIDEMIOLOGICAL STUDIES OF NEMATODES IN FISHES

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Three hundred fresh water fishes of six species were collected from six different fish farms of Lahore for the prevalence study of nematodes. Out of 300 fishes sample examined, 12 were found to be infected with the helminthes, majority were isolated from the stomach and intestines. The following species of nematodes were recorded; *Capillaria* spp. and *Eustrongylides* spp. The overall prevalence of intestinal nematodes was recorded as 4% (12/300). The monthly prevalence of nematodes was recorded and the range of monthly incidence was 0-8%. The highest prevalence of nematodes was 8% (4/50) during March, while the lowest prevalence was noted in June 0%. *Sperata sawari* showed the maximum infestation of nematodes of 8% (4/50). Whereas *Hypophthalmichthys molitrix* showed the minimum prevalence of nematode of 0%. The prevalence of different nematode in a particular fish species was recorded. *Capillaria* spp. showed overall prevalence of 3.33% whereas *Labeo rohita* and *Channa marulius* showed maximum of 6% *Capillaria* spp. infestation. Similarly overall infestation of *Eustrongylides* sp. was recorded as 1.33% and maximum prevalence was showed by the *Sperata sawari* and *Hypophthalmichthys molitrix* 4%. The nematode intensity might be linked with the genetic makeup, intestinal vigor, and other managerial and environmental factors.

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### **ELISA-BASED SURVEILLANCE OF GOAT FASCIOLIASIS FROM SLAUGHTER HOUSE OF FAISALABAD**

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Fascioliasis is an economically important helminth infection of small and large ruminants which causes significant losses worldwide. Early diagnosis is helpful for control and treatment of disease. In continuation with our previous studies, this report describes the results of serosurveillance of goat Fascioliasis in Faisalabad slaughter house through indigenous ELISA technique using excretory/secretory Ag. Blood samples collected from Faisalabad slaughter house during May, 2013 to October, 2013 in gel clotted vacutainers were processed for separation of sera which were screened for anti-Fasciola Ab using BioRad Microplate Reader at 450 nm wavelength. Out of 630 sera, 69 (10.90%) were found positive for Ab against *Fasciola* spp. Monthly distribution of Ab was highest in September (19.04%) and lowest in June (5.71%). The most susceptible breed of goat was non-descript (13.80%) followed in order by Teddy (11.88%) and Beetal (7.84%). Females were statistically more susceptible to fascioliasis than males. However, age was not found having any statistical association with the sero-positivity. The development of ELISA using indigenous antigens is more sensitive and specific test and it may be recommended for use in nation-wide seroepidemiological surveys for fasciolosis at cheaper cost.

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### **DOES *HAEMONCHUS* *CONTOURS* REALLY HYPOBIOSE DURING WINTER IN METROPOLITAN FAISALABAD?**

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*Haemonchosis* is parasitic disease of ruminants round the globe caused by *Haemonchus* (*H.*) *contortus* (Strongylida: Trichostrongylidae). This is known to be a serious health threat to small ruminant population in terms of production losses, high morbidity, mortality, treatment cost and control measures. The objective of this presentation is to provide a point prevalence of small ruminant haemonchosis in slaughter houses of Faisalabad metropolitan during the winter season 2013-14. Small ruminants including sheep and goats were subjected to antemortem inspection in the abattoir. The abomasa of slaughtered animals will be collected and brought to the Department of Parasitology for isolation of worms, their recovery and quantification through standard

parasitological protocols. Faecal samples of the respective animals will also be collected for routine coprological examination through flotation technique followed by Mc Master egg counting chamber and coproculture. The study is underway and so far, 14 out of 17 abomasa and 19 out of 30 faecal samples were found positive for adults and eggs of *H. contortus*, respectively. The female worms were successfully cultured using conventional culture approach to procure L3. The study will continue till February 2014 to provide data on the ecobiology of *H. contortus* during the winter season which is usually rendered as the hypobiotic phase of this parasite. The availability of worms as well as eggs in the host population during the winter season further alarms the scientific community for identifying appropriate control strategies of this parasite.

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**POINT PREVALENCE OF TICKS IN SELECTED LIVESTOCK POPULATIONS OF KHYBER PAKHTUNKHWA AND GILGIT BALTISTAN, PAKISTAN**

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Ticks (Chelicerata: Acari) are obligate blood imbibing ectoparasite of wide range of mammals including humans which are dispersed globally. Ticks cause high economic losses to livestock industry through direct and indirect ways. In continuation with our previous studies, the present investigation was conducted during the summer season 2013 to determine the point prevalence of ticks infesting the free ranged livestock population of selected regions of KPK and Gilgit-Baltistan (GB). Of 813 animals screened during the survey, an overall prevalence of tick was found 75.03 % with highest prevalence in district Haripur (85.58%) followed by Gilgit (83.10%), Mansehra (81.14%), Batagram (81.05%), Shangala (77.78%), Kohistan (75.38%), Diamer (72.28%) and Astor (32.22%) districts. *Hylomma anatolicum anatolicum* and *Rhipicephalus microplus* were the tick species identified on the basis of their morphological features using a standard identification keys. With respect to animal species sheep (81.47%) was the most prone followed in order by cattle (77.91%), goats (72.05%) and buffaloes (58.49%); whereas, yak population was found free from tickosis. Prevalence of ticks was optimum in young stock (85.67%) and females (80.33%) than adults (66.44%) and males (67.27%), respectively. A huge number of animals found positive for tick infestation even at higher altitudes of Pakistan alarms the scientific community for appropriate control strategies to be adopted in those areas too. Moreover, the animals of KPK and GB may also be presumed to carry tick-borne infections which will be confirmed after complete blood analyses. The tick vectors collected through this survey will be subjected to molecular protocols for identification of various probable pathogens which is underway. Another



point of concern for scientific community could be the provision of eco-biologically compatible environment in higher altitudes which may fairly be correlated with the climate change.

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### THE PREVALENCE OF ENDOPARASITES IN CHICKENS (BROILER) IN BAHAWALPUR

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A study was conducted to investigate the prevalence (%) of endoparasites in chickens (broiler) in Bahawalpur from January to June 2013. Different sampling techniques were used for processing of faecal samples. Of 400 faecal samples examined, 280 (70%) were found infected. Protozoan Parasite *Eimeria* sp. and two Nematode parasites *Syngamus trachea* and *Ascaridia galli* were recorded. The *Eimeria* sp. 47% was found to be high prevalent followed by *Syngamus trachea* 10.5% and *Ascaridia galli* 12.5%.

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### EPIDEMIOLOGICAL INVESTIGATION OF BABESIOSIS IN CATTLE POPULATION OF DISTRICT KHANEWAL, PUNJAB, PAKISTAN

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Babesiosis is a tick-borne haemoprotzoan disease of large ruminants caused by a number of *Babesia* spp. but the main species in bovine are *Babesia* (*B.*) *bovis* and *B. bigemina*. Biological transmission is mainly vectored by ticks of genus *Rhipicephalus*. In this study, a cross-sectional survey was conducted in selected cattle population of district Khanewal, Punjab, Pakistan, in order to determine the epidemiology of cattle babesiosis. Of the 1488 animals screened through conventional optical microscopy of Giemsa-stained blood films, overall prevalence of babesiosis was calculated as 21.37% (318/1488). A pre-designed questionnaire was used to determine the association of age, host and environment related factors with the disease prevalence. The prevalence of babesiosis was higher in calves and females than adults and males, respectively. Friesian cattle were found more prone to babesiosis as compared to cross-bred and Sahiwal

cattle. Summer season was found with the highest disease prevalence followed by autumn, spring and winter. Infection menace was recorded higher in tethered animals than free moving and closed housing system than semi-close and open housing systems. Poor hygienic animals were more at risk than those having good hygienic measures at farms. Animals kept on un-cemented floor were chronicled more in danger of babesiosis than those animals kept on partially cemented and cemented floor. The data will not only be helpful for the dairy farmers to modulate farming practices but also for the policy and decision makers to control the nuisance in the livestock population of the district. Further studies are proposed to determine the prevalent *Babesia spp.* in the study area by using modern diagnostic techniques.

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**TICK INFESTATION IN CAMEL OF DESERTED ECOSPHERE IN SINDH,  
PAKISTAN**

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Field collections were made from camel in various localities of Therparker desert (Mithi, Nangarparker, islamkot and Chachro) aimed at developing base line data on tick infestation. A total of 62 camels were examined and checked for tick infestation. Of 62 infested camels, 43% were found infested with ticks. Gender-wise data revealed that, females were more susceptible (70%) when compared with male that showed 66.66 % camel infested with ticks. Data on age-wise infestation depicted that, the young camels (77%) are more prone to infestation as compared to adult (65%). Further investigations are under way.

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**CENTRORHYNCHUS SCANENSE LUUNDSTROM, 1942 IN INDIAN BUTTON  
QUAIL (*TURNIX SYLVATICA*) (DESFONTAINES) FROM KARACHI**

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A survey was conducted to record acanthocephalan from birds of Karachi, Sindh, Pakistan. Thirty Indian button quail purchased from Empress Market, Karachi revealed that 6 birds were infected with acanthocephalan. The thirteen parasites recovered were fixed in A.F.A. solution (a solution of ethyl alcohol formalin, acetic acid in the ratio of 92:5:3) for 24 h, washed with 70% ethanol, stained with Mayer's carmalum, dehydrated

in graded alcohol, cleared in clove oil, rinsed with xylene and mounted permanently in Canada balsam. Drawings were prepared with the aid of camera Lucida. Morphometric studies specify that the Acanthocephala were *Centrorhynchus scanense* Lundstrom, 1942 which are being redescribed. This is the seventh species of the genus *Centrorhynchus* Luhe, 1911 from Pakistan.

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**A NEW SPECIES OF TREMATODE *ECHINOSTOMA KHAIRPURENSIS* N.SP.,  
FROM BLACK-WINGED STILT *HIMANTOPUS HIMANTOPUS* OF KHAIRPUR  
DISTRICT OF SINDH, PAKISTAN**

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During current studies on helminth fauna of birds in Khairpur district of Sindh province of Pakistan, a total of 106 birds were examined. Live birds were collected from different localities of study area and brought to Parasitology Laboratory of Department of Zoology. Birds were anesthetized with chloroform and dissected. All viscera were separated in Petri dishes and examined under dissecting microscope. During the examination of gut contents, a total of 71 trematodes belonging to the genus *Echinostoma* Rudolphi, 1809 were collected from intestine of Black-winged stilt *Himantopus himantopus*. Trematodes were collected and processed according the standardized techniques. On the basis of shape and body size, number of collar spines, size of eggs and other morphological characteristics, a new species *Echinostoma khairpurensis* is proposed. Name of the new species refers to the locality of host.

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**HELMINTH PARASITES OF BANK MYNA *ACRIDOTHERES GINGINIANUS*  
(PASSERIFORMES: STURNIDAE) OF KHAIRPUR DISTRICT, SINDH,  
PAKISTAN.**

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Helminth fauna of birds of Khairpur district of Sindh province, Pakistan are very poorly known. During current studies of helminth parasites of Bank Myna *Acridotheres ginginianus* (Passeriformes: Sturnidae) of Khairpur District, a total of 15 hosts were examined. Live birds were collected from different localities of study area and brought to Parasitology Laboratory of Department of Zoology. Birds were anesthetized with chloroform and dissected. All viscera were separated in Petri dishes and examined under dissecting microscope. Out of 15 Bank Myna, 80% were infected with helminth parasites.

Highest 75% was recorded for nematodes (88% Fillaroid worms and 12% rest of nematode), followed by cestode and trematodes 50%. Minimum 34% was recorded for acanthocephalan. Helminths collected were processed according to the standardized techniques and will be identified with keys and literature.

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**DESCRIPTION OF NEW ACANTHOCEPHALAN *POLYMORPHUS PLATYRHYNCHOSI* N.SP. (ACANTHOCEPHALA: POLYMORPHIDAE) IN MALLARD *ANAS PLATYRHYNCHOS* (ANSERIFORMES: ANATIDAE) IN SINDH, PAKISTAN**

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In result of ongoing helminthological studies of Mallard *Anas platyrhynchos* of Kamber- Shahdadt District of Sindh province, Pakistan, a total of 12 birds were captured from different localities of the study area. During examination of gut contents and visceral organs, a total of 86 specimens belonging to genus *Polymorphus* Lühe, 1911 were collected from large intestine of hosts. Present species differs from its close allies in body shape, number of longitudinal rows of hooks, number of hooks in each row, size of hooks and size and shape of different organs. On the basis of major diagnostic differences between present species and its close allies, a new species *P. platyrhynchos* is proposed.

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**SPECIFIC IDENTIFICATION OF NEMATODES OF THE GENUS *TELADORSAGIA* BASED ON MOLECULAR GENETIC MARKERS**

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Nematode *Teladorsagia circumcincta* (Stadelmann, 1894) and *T. trifurcata* (Ransom, 1907), belonging to the genus *Teladorsagia* Anreeva et Satubaldin, 1954 of the family Trichostrongylidae Leiper, 1912, widespread parasites of ruminants in Uzbekistan. They are located in the abomasums and, in some cases, they are found in the small intestine. Intensity of infection reaches thousands of individuals in one animal. Our objective was to a molecular identification based on ITS-1+5.8S+ITS-2 sequences of the ribosomal DNA of *T. circumcincta* and *T. trifurcata* from sheep, obtained from different regions of Uzbekistan, with the purpose of identifying differences between and obtaining additional data on the rDNA. Mature individuals w both species were collected from the mucus of sheep abomasums in slaughter houses Bukhara and Namangan regions of

Uzbekistan. Nematodes were washed with the physiological solution and the specimens of nematodes were conserved in 70% ethanol and their taxonomic affiliation was determined by a complex of morphological features. Were investigated partial sequences of the internal transcribed spacer (ITS) rDNA of *T. circumcincta* and *T. trifurcata*, which are presumably constitute two morphologically distinct variants of a single species. The length of the sequences was 781 pairs of nucleotides. The level of difference discovered between the ITS-1+5.8S+ITS-2 sequences of *T. circumcincta* and *T. trifurcata* was 0, 38 % and did not exceed it in samples taken from the *T. circumcincta* morph. At the same time, the ITS difference level was substantially higher among *T. circumcincta* and other Trichostrongylidae species. The level of difference was 7, 4 % between *T. circumcincta* and *O. ostertagi*, and 5, 2 % between *T. circumcincta* and *M.marshalli*. Obtained data confirm that *T. circumcincta* and *T. trifurcata* are morphologically distinct variants of a single species.

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#### REPRODUCTION AND PATHOGENICITY OF *MELOIDOGYNE INCOGNITA* ON FIVE CHILLI CULTIVARS

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Root-knot nematodes are the most widely distributed, destructive plant pathogens and cause colossal yield loss. The losses are considered to be influenced by inoculum levels and type of cultivar. In the present studies effect of different inoculum levels (0, 500, 1000, 2000, 5000) of *Meloidogyne incognita* was investigated on five chili cultivars ( High Fly, PV-VI, Revival, Kot Sultan, Skyline II). All the inoculums levels varied significantly in causing reductions in growth parameters. The reduction in growth parameters increased with an increase in inoculum levels showing a direct relationship between inoculum levels and growth parameters. The inoculum levels also affected nematodes infestations. Number of galls and egg masses increased with an increase in inoculum levels; being minimum at lowest level and maximum at highest density. On the other hand, fecundity and rate of nematode build up was found to be the maximum at the lowest level. As the level increased significant increase in these parameters were observed. Inoculum levels behaved differently on different cultivars. Maximum reductions in growth parameters, nematode infestation and reproduction of *M. incognita* were observed in case of Highfly. The cultivar Skyline-II was the least affected. None of the cultivars was found to be immune or resistant against *M. incognita*. The cultivar Skyline-II was found to be susceptible and the remaining cultivars were highly susceptible.

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**PREVALENCE OF HELMINTH PARASITES OF *GALLUS GALLUS DOMESTICUS* (GALLIFORMES: PHASIANIDAE) OF KHAIRPUR DISTRICT, SINDH, PAKISTAN**

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During the current studies of helminth parasites of Domestic Chick *Gallus gallus domesticus* (Galliformes: Phasianidae) of Khairpur District, a total of 9 hosts were examined. Live birds were collected from different localities of study area and brought to Parasitology Laboratory of Department of Zoology. Birds were slaughtered and all viscera separated in Petri dishes and examined under dissecting microscope. Out of 9 Domestic Chick, 89% were infected with helminth parasites. Highest 100% was recorded for cestodes, followed by 50% for nematodes and trematodes. Helminths collected were processed according to standardized procedure and shall be identified with keys and literature.

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**PARASITIC INFECTION IN IMPORTED ORNAMENTAL FISH FANTAIL, A VARIETY OF GOLDFISH, *CARASSIUS AURATUS* L.**

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The aim of the present study was to investigate parasitic infection in an imported ornamental fish fantail, a variety of goldfish, *Carassius auratus*. Thirty fantail fishes were purchased from commercial pet shop in Lahore. The fishes were examined for parasitic infection according to standard parasitological procedures in Fish Disease and Health Management Lab. In total seven different species of parasites belonging to four groups; protozoans, monogenean, digenean and crustacean were found. *Trichodina* sp. infection on skin and fins was 20% (mean intensity 9.5) *Ichthyophthirius multifiliis* infection on gills and fins was 43.3% (mean intensity 20.72) and 30% (mean intensity 29.25) respectively. *Ichthyophthirius multifiliis* were of large size and crescent shaped macronucleus was very clear. *Tetrahymena* sp. infection was 10%, (mean intensity 98). The gills were infected with *Dactylogyrus* sp. and infection was 100% (mean intensity 29.3). Whereas, *Gyrodactylus* sp. infection on skin of fish was 70% (mean intensity 11.1). *Argulus foliaceus* infection was 26.60% and mean intensity was 6.0. Metacercariae of an unidentified digenean were also found encysted on gills. The infection was 66.60% (mean intensity 20.4). The *dactylogyrus* infection was most serious which caused severe damages to gill filaments and secondary lamellae. Other parasites seem to be less pathogenic to these fishes. The import of diseased and infected fishes may

by strictly checked and regulated by the authorities to control transmission of parasites into Pakistan.

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### **MALIGNANT THEILERIOSIS: A THREAT TO SMALL RUMINANTS OF PAKISTAN**

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Malignant theileriosis is a fatal disease of small ruminants caused by a haemoprotozoan known as *Theileria* (*T.*) *lestquardi* which is morphologically and biologically similar to *T. annulata* (a causative agent of bovine tropical theileriosis). The risk of malignant theileriosis is same as that of bovine tropical theileriosis, because, both of these pathogens share the same vector. Malignant theileriosis is prevalent in Sudan, Algeria, Turkey, Iran, Iraq, Middle East, China and India. This disease is responsible for mild to severe morbidity and mortality leading to huge economic losses in small ruminants. *Hyalomma* spp. is prevalent in tropical and sub-tropical countries of the world. In Pakistan, *Hyalomma* species are abundantly prevalent and reported to transmit theileriosis in all domestic livestock species across the country. The animals once infected with theileria protozoans, remain carrier for years even treated and remain the continuous source of infection for ticks which can spread the disease to other animals. *Theileria* species have a complex life-cycle, involves both, the transmitting invertebrate tick vector, in which sexual reproduction by sporogony takes place, and the vertebrate host, in which asexual reproduction by schizogony and merogony occurs. The diagnosis is usually based on clinical signs and symptoms whereas conventional laboratory diagnosis for host blood (Giemsa's staining), for salivary glands (Feulgen or Methyl green pyronin staining) and serological test (IFAT, ELISA) are used. However, these techniques cannot differentiate *T. annulata* and *T. lestquardi*. The molecular techniques of polymerase chain reaction (PCR) and reverse line blot hybridization (RLB) for identification of *Theileria* spp are more specific and sensitive. Unfortunately in Pakistan, malignant theileriosis has not been studied well, however, there are some reports of ovine theileriosis based on traditional optical microscopy. Not only *T. lestquardi* but, *T. luwenshuni*, *T. uilenbergi*, *T. ovis* and *T. separate* are also pathogenic for small ruminants. The objective of this presentation is to highlight the issues related to capacity of research in the area of malignant theileriosis in the small ruminant population of Pakistan which count to approximately 91.5 million heads among the livestock population. The first step towards the possible areas could be surveillance of disease from a narrow scale to wide scale.

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**PREVALENCE OF ASCARIDIA COLUMBAE IN HYDERABAD AND  
JAMSHORO DISTRICTS OF SINDH**

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During an investigation on helminth parasites of birds fourteen *Calumba livia* (Pigeon) were purchased from local market in Jamshoro and Hyderabad Districts, Sindh Pakistan. The birds were anaesthetized/autopsied and examined for helminth parasitic infection. Out of fourteen four were found infected with nematodes. The specimens were studied in detail and identified as belonging to genus *Ascaridia calumbae* (Gmelin, 1780). The genus is characterized by having, stout worms, usually with three lips, esophagus is muscular with caudal bulb. Ventriculus is prominent. Male: are distinguished by the ventrally curved tail, two copulatory spicules. Female: vulva situated is near the end of the first third of the body.

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**PREVALENCE OF HELMINTH PARASITIC INFECTIONS IN RATS (*RATTUS  
RATTUS*) IN DIFFERENT AGRICULTURAL FIELDS OF DISTRICT SWAT,  
PAKISTAN**

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During the study a total of 269 rats were trapped from different agricultural fields of district Swat, Pakistan. The viscera were collected after autopsy examination of the rats and preserved in 70% alcohol and then brought to VPCI, SARC/PARC, Medical Zoology Laboratory for helminths investigation. The viscera were then opened and the organs like stomach, small intestine, large intestine, liver and lungs were opened longitudinally. These organs were found to be infected with helminth parasites: Nematodes, Cestodes and Trematodes. Nematodes (round worms) were isolated and placed in a Petridish containing normal saline solution, then preserved in 70% alcohol and glycerin solution for detail microscopic examination while the Cestodes and Trematodes were slightly pressed b/w two glass-slides, kept in FAA solution for 24-hours for fixation then dehydrated with graded series of alcohol, stained with Mayer's Carmalum, cleared with clove oil, rinsed with xylene and mounted with Canada balsam. The over all prevalence in order to their intensity of helminth parasites is as: *Syphacia* sp. 13 (4.83%), *Aspiculuris* sp. 13 (4.83%), *Heterakis* sp. 12 (4.46%), *Hymenolepis*



*swatensis* sp.n. 9 (3.34%), *H.diminuta* 8 (2.97%), *Lutziella microacetabularae* 4(1.48%), *Hymenolepis fusa* 4 (1.48%) and *Lutziella swatensis* sp.n. 1 (0.37%) were reported. No considerable difference in the prevalence of parasites was noted b/w areas, crops, crop stages and sex of the host while adults were found more infected than sub-adults.

### **HISTOLOGY OF INTESTINE OF CROW INFECTED WITH *CENTRORHYNCHUS AMINI* KHAN *ET AL.*, 2012**

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Observations on the histology sections of acanthocephalan (*Centrorhynchus amini* Khan *et al.*, 2012) to crow (*Corvus splendens* Vieillot) was studied in detail. Severe damage occurred to the whole thickness of the intestinal wall including villi, crypt glands and muscular layer. Large lesions were observed between the crypt glands and muscular portion which contained to lower part as penetration track of acanthocephalan. The damaged crypt glands appeared as a mass of cells with no proper structure. Here muscular fibres were also adversely affected and produced large spaces.

### **STUDY OF *ISOPARORCHIS* SP. SOUTHWELL, 1913 (FAMILY: *ISOPARORCHIIDAE*) PARASITE FROM THE FRESH WATER FISH *WALLAGO ATTU* IN SINDH, PAKISTAN**

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Eight *Wallago attu* were purchased from the market of District Jamshoro, Sindh, Pakistan. The fishes were brought to the Parasitological laboratory, University of Sindh, Jamshoro, these were anesthsized autopsied and examined for helminth parasitic infections. Out of eight fishes, six were found infected. Twenty one specimens of parasite were recovered from the swim bladder. The worms were mounted permanently according to standard procedures; and identified belonging to the genus level *Isoparorchis* sp. Southwell, 1913 Syn. *Leptolecithum* Kobayashi, 1915. The genus is characterized by having: body is very large, foliate and translucent when extended, unarmed, with margins turned over ventrally. Oral sucker is subterminal, surmounted by preoral lobe. Pharynx is contiguous with oral

sucker. Esophagus is very short. Ceca with stomach portion at commencement, running sinuously to posterior extremity. Acetabulum is in anterior half of body. Testes are posterolateral to acetabulum. Vesicula seminalis is tubular, convoluted anterior to acetabulum. Hermaphroditic sac is pre-acetabular. Genital pore is behind intestinal bifurcation. Ovary is winding, submedian, near posterior extremity. Vitellaria median, immediately behind ovary, consisting of several subdivided branches overreaching ceca laterally. Laurer's canal forming receptaculum seminis at its origin. Uterus dorsal, forming transverse loops across ceca; metraterm is well differentiated, penetrating hermaphroditic sac. Excretory stem sigmoid, dilated posteriorly; arms serpentine, united dorsal to pharynx. Parasitic in swim bladder of fresh water fishes.

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#### **SENTINEL SURVEILLANCE OF FASCIOSIS IN CATTLE AND BUFFALOES OF TEHSIL NASIRABAD, AZAD JAMMU AND KASHMIR**

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Fasciolosis is a parasitic disease of ruminants, caused by two main species of genus *Fasciola* i.e. *Fasciola* (F.) *hepatica* and *F. gigantica* and these are commonly documented as liver flukes. It causes massive economic and production losses to livestock industry. The disease is known to be epidemic in marshy areas round the globe. Nasirabad (Study area) is marshy area which is conducive for the propagation of parasite. Passive surveillance of fasciolosis in cattle and buffalos of the study area has been carried out for the period of one year i.e. from November, 2012 to October 2013. A total of 88 cattle and buffalos which were suspected for fasciolosis had been screened through direct and indirect coprological technique (flotation). Results of present study depicted that the disease is epidemic in the area as the overall prevalence was 88.64% (78/88) with higher prevalence of *F. gigantica* 50% (44/88) as compared to *F. hepatica* 38.64% (34/88). Buffalo 85.83% (23/24) were found more prone to fasciolosis compared to cattle 75% (48/64). Age and sex was not found statistically associated with the disease prevalence; whereas, prevalence of fasciolosis observed was optimum in warmer months of the year. This is probably the first report on fasciolosis from the study area. Therefore, complete randomized large scale future investigation to be planned to determine the factor associated with this high prevalence of fasciolosis in the study area especially and AJK generally.

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**A NEW SPECIES OF THE GENUS *PALLISENTIS* VAN CLEAVE, 1928, IN A  
FISH *LABEO ROHITA* (HAM.) FROM SINDH**

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In a survey of helminth parasites of fish (*Labeo rohita* Ham.) two male and two female specimens belonging to the genus *Pallisentis* Van Cleave, 1928 were recovered from the intestine. Usual techniques for collection and preservation of the acanthocephalan was employed, while whole mounts were stained with Mayer's carmalum and cleared in xylol. The present species has no resemblance with any previously reported species of the genus, thus is regarded as a new species with the name *P. orientalis*.

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**SECTION - V****FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY,  
MARINE BIOLOGY****1. ECOLOGY AND ENVIRONMENTAL POLLUTION****ENVIRONMENTAL POLLUTION AND ITS IMPACT ON NATURAL  
RESOURCES**

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Marine pollution arising from various anthropogenic substances is constantly a threat to marine ecosystem. The major pollutants entering into the coastal environment are oil, sewage, garbage, pesticides, toxic chemicals, heavy metal, radioactive waste, thermal pollution and nutrients. Emission of hazardous waste from industrial activity is causing serious health problems and deteriorating the environmental conditions. The continuous discharge of industrial wastes including heavy metals and other toxic substances and the indirect release of nitrates, phosphates and pesticide products often result in toxic accumulations in the marine food chain. Pesticides have direct effect on photosynthesis in microalgae, an important link in the energy transformation to higher trophic levels. Marine consumer organisms, such as artemia, mysid, fish juveniles, appear to be highly sensitive to pesticides and have low LC50 values. Increasing coastal pollution and high sensitivity of marine organisms to these pollutants may have direct effect on the fish diversity and fishery industry.

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**ROLE OF WEEDS IN CREATING AGRO-ECOLOGICAL STABILITY**TAHIRA RUBY,<sup>1</sup> SHAHNAZ AKHTAR RANA<sup>2</sup> AND ALEEM AHMAD KHAN<sup>3</sup><sup>1</sup>*Department of Life Sciences, The Islamia University of Bahawalpur.*<sup>2</sup>*Department of Zoology and Fisheries, University of Agriculture, Faisalabad.*<sup>3</sup>*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.**Corresponding author: Email: [tahira.ruby@iub.edu.pk](mailto:tahira.ruby@iub.edu.pk)*

We devised a study to ascertain the role of weeds in agro-ecosystem. Therefore, we made seasonal cataloguing of the line data on multiple crops *i.e.*, sugarcane, fodder, wheat and mustard to see crops' viability and role of weeds' diversity in preventing insect outbreaks by reducing crop productivity losses. We found some fifteen species of weeds

include 11 weed species were of broad-leaved category while four were of pointed-leaves. The arthropod-fauna include insect pest-species from Orthoptera, Hemiptera and Lepidoptera that used weeds as priority food. Besides that, some specific zoophagous insect-predators belonging to orders Odonata, Coleoptera, Hymenoptera and Araneae were documented on similar weeds, for want of food, shelter and egg-laying. In the light of our observations, we conclude a significant role of weeds in a crop-system may support other essential life forms in creating ecological balance.

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### **INVESTIGATION OF POTENTIAL SYNERGIES BETWEEN BIODIVERSITY AND FARM PRODUCTION SUSTAINABILITY**

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Agriculture is by far the significant important sector for Pakistan's economy and 99% of exports are directly or indirectly based on agricultural commodities. It not only provides employment opportunities to more than 60% of the country's population but also food to more than 162 million people and supports earning of foreign exchange. Land resources relative to population are limited and have declined over current decades. The hypothesis that if agro-ecosystem is modeled after natural ecosystem, the agro-ecosystem should exhibit many functional attributes and processes that stabilize the system. A detailed study was conducted to determine floral and invertebrate faunal diversity of the cropland. The relationship of the various synchronous and synergistic taxa of crop fields were estimated along with system cost effectiveness. Five different crop-weed complexes, including Wheat, sugarcane, cotton, Rice and fodder and 39 weed species present in these crops were sampled throughout study duration. A total of 83,543 specimens representing 736 species were captured from cropland of different zones *e.g.*, Mixed-crop zone (MCZ), Cotton-wheat zone (CWZ), Rice-wheat zone (RWZ). All of these species belonging to nine different orders namely, Microcoryphila, Opisthophora, Haplotaenidia, Araneae, Chilopoda, Odonata, Orthoptera, Mantodea, Dermaptera, Blattaria, Hemiptera, Coleoptera, Diptera, Lepidoptera, Hymenoptera, Eupulmonata, Neuroptera, Isopoda, Collembola and Pulmonata. Whole fauna was classified into groups on the basis of their trophic status in the field like Prey/Pest, Predators, Omnivores, Detritivores or Scavengers. Prey/pest having herbivore feeding niche were 57.60% and was most dominant group. Predators were second highest group in total cropland fauna with its total percentage 31.8 with maximum contribution of fodder crops as 8.78% followed by sugarcane 6.97%. The comparison of these systems revealed MCZ (Mixed-crop zone) wheat farms as best with respect to crop production and net biomass production, whereas, recyclable biomass (weeds+ invertebrates) was highest in RWZ. These investigations of these synergies may effectively be utilized for sustainable agro-ecosystem.

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**THE BEHAVIOR OF EARTHWORM POPULATIONS AND THEIR EFFECT IN THE SOIL FERTILITY WITH RESPECT TO CLIMATIC FACTORS FROM SELECTED FIELDS**

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The influence of earthworms on the soil texture, physiology, fertility and the effect of cropping practices along with climatic factors on the earthworm diversity and abundance were investigated in the four cropping fields cotton (*Gossypium hisutum*), wheat (*Triticum aestivum*), sugarcane (*Saccharum officinarum*) and maize (*Zea mays*). Samplings were done after every month with five replicates. Up to thirteen species belonging to seven genera were harboured from the selected sites. Overall, about 85.08% of earthworms were belonging to genus *Pheretima*, followed by *Dendrobaena* (7.95 %) and *Ramieella* (5.5%) having significant effects on soil topography. The number of earthworms gradually decreased from the edge of water channel towards the center of the fields. Seasonal/ climatic factors showed significant influence on the diversity and abundance of the earthworms ( $p = 0.01$ ,  $p = 0.05$ ). Genus *Pheretima* fairly found throughout studied period/ session. *Dendrobaena* found in the dry and cold months of December through February. The casts and burrows were also steadily decreased from the edges of the water channel to the center of the fields and as increased depth of the top soil to the subsoil ranging from one to three ft. There was a statistical correlation between soil fertility and abundance of earthworms.

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**BREEDING BIOLOGY OF HOUSE CROW (*CORVUS SPLENDENS*) IN THE VICINITY OF HAZARA UNIVERSITY GARDEN CAMPUS, MANSEHRA, PAKISTAN**

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House Crow (*Corvus splendens*) is passerine bird originated in sub-continent ranging in size from large to medium small. Study on the nesting House Crow was conducted in the Garden Campus of Hazara University, Mansehra (area 125 acre) during breeding season June to September 2013. Tree characteristics including Tree height, Diameter at breast height and Clear bole height, Canopy Spread, and Circumference of tree trunk were measured by measuring tape and formulae. Nest characteristics including nest height, weight, mean diameters, nest depth, nest cup diameter and nest cup depth

were measured by small measuring tape. The egg parameters like egg length and breadth were calculated by using Vernier Calliper with 0.1 mm least count. The egg volume was calculated by using Hoyt (1979) formula *i.e.*  $V = 0.51 \times L \times B^2/1000$ . And egg shape index is calculated by dividing length/breadth. The data was analyzed by (ANOVA) Analysis of Variance. The results showed that House Crow nested on 4 different tree species viz *Melia azedarach*, *Pinus roxburgii*, *Platanus orientalis* and *Populus euphratica*. The tree species *P. orientalis* was favoured nesting tree. Mean tree and nest height was 14.8 and 11.8 m, respectively. Mean nest density of House Crow on research was 2.45 nests/acre. Mean diameter of nest and nest depth was 42.3 and 19.3 cm, respectively. Mean nest cup diameter and nest cup depth was 15.6 and 8.3 cm, respectively. Mean nest weight was 1.4 Kg. Mean clutch size was 4.0. Mean egg volume was 13.34 cm<sup>3</sup>, egg length 38.6 mm, breadth 26.0 mm and egg shape index 1.42. Out of total 51 nests, 29 found to be active. Breeding season extended early June to early September; egg laying occurred early June to mid-July; hatchlings occurred from late June to early August and fledglings was observed from mid-july to early September. Reproductive achievements and failures were also recorded. Main reasons for reproductive failures were unhatched eggs, predators, poor nest construction, bad weather and observer's disturbance. Eggs hatching success rate was 0.551 and nest success rate was 0.656. It is concluded that House Crow is a tree nester and favors nesting in the trees taller in length with a dense canopies so to keep away from human harassment but near human surroundings.

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**ASSESSMENT OF A MECHANICAL METHOD FOR INHIBITING ROSE-RINGED PARAKEET (*PSITTACULA KRAMERI*) IN AGRO-ECOLOGICAL SYSTEM OF FAISALABAD, PAKISTAN**

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The Rose-ringed parakeet (*Psittacula krameri*) undoubtedly, a serious kind of pest among the cultivations in Pakistan, causes a substantial damage to both the cropped and horticultural interests. The depredation annually brings about economic losses to the farmers and the country, in particular in the guarded situations. The present research is, therefore, aimed at devising a sustainable strategy at the mature and post harvest stages of wheat and maize through the use of wind powered hawk eye rotator which is a bird scaring device. Study was conducted during evening hours, the time when the infestations are far more by the birds on the cropped areas. Present information on the foraging profiles of the rose-ringed parakeet in the unguarded situations. The unguarded situation is the one in which there is no protection to the field crops. The observations were made for five continuous days, while for the next five days, the protection was provided in the form of the hawk eye rotator. It was evident that in the earlier stages, very few parakeets

visited the field crop. However, with the passage of time they kept on increasing. Same situation was with regard to leaving the parakeet from crop. The guarded situation using the hawk eye rotator, placed in the center of the field, for inhibiting the rose-ringed parakeet movements, sufficiently, reduced the visitation in the field. It meant that, the installation of a mechanical device, primarily considered as a bird repellent, proved to be advantageous to the field crop. The movements were there but fairly reduced as compared to the one in the unguarded stage. The damage intensity also reduced substantially. It is also worth pointing out that the number of visits of parakeet to the field following the application of the wind powered hawk eye, also reduced. There is an utmost need to reduce its damage by using the non-chemical devices, so that efficient and environment friendly management can be achieved.

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#### **DNA FRAGMENTATION COMPARISON IN DIFFERENT FISH ORGANS AS POLLUTION ASSESSMENT TOOL**

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Intense pollution being contributed by human is not only threatening irrigation and ground waters but also terrestrial and aquatic life. The present study was planned to assess the impact of habitat on the quality of DNA and contribution of weight in % DNA fragmentation to assess the pollution level in different fish organs (kidney, liver, gills, muscle) of (*Wallago attu* and *Labeo rohita*) collected from River Chenab and Fish Seed Hatchery, Satiana Road, Faisalabad as polluted and control samples, respectively. The fish samples were processed in the Research laboratory, GC University, Faisalabad. After morphometric measurements, each fish specimen was dissected to collect kidney, liver, gills and muscle specimens. Percent DNA fragmentation was examined by spectroscopic method. In case of *Wallago attu*, in case of kidney the %DNA Fragmentation ranged between minimum value of 62.75 whereas maximum value was 75.12. The %DNA Fragmentation ranged between minimum value of 57.17, 56.83 and 44.21 whereas maximum value was 63.57, 61.91 and 52.37 in liver, gills and muscle respectively. In case of kidney of control samples it ranged between minimum value of 45.12% whereas maximum value was 47.29%. The %DNA fragmentation ranged between minimum value of 35.12, 31.21 and 27.21 whereas maximum value was 38.61, 36.75 and 35.12 in liver, gills and muscle, respectively. There was no % DNA fragmentation observed in different fish organs of control samples when compared with experimental samples. In case of *Labeo rohita*, in case of kidney the %DNA Fragmentation ranged between minimum value of 28.17% whereas maximum value was 64.25%. The %DNA Fragmentation ranged between minimum value of 26.13, 24.17 and 22.12 whereas



maximum value was 59.64, 58.40 and 50.19 in liver, gills and muscle, respectively. In case of kidney of control samples it ranged between minimum value of 20.94% whereas maximum value was 40.82%. The %DNA Fragmentation ranged between minimum value of 20.21, 20.21 (gill & muscle) whereas maximum value was 35.99, 35.62 and 34.62% in liver, gills and muscle, respectively. There was an increasing trend of %DNA Fragmentation in all the fish organs and different weight categories, organs showed highly significant effect ( $P < 0.01$ ). When correlation between % DNA fragmentation weight and different organs regarding %DNA fragmentation in both species was observed. The weight has a positive and highly significant ( $P < 0.01$ ) effect on %DNA fragmentation in kidney, liver, gills and muscle.

#### POPULATION TRENDS OF BIRDS AT TAUNSA BARRAGE WILDLIFE SANCTUARY

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Population trends learning, which identifies potential threats, is an important tool for conservation programs in rare as well as in common birds species. A study was conducted to estimate annual decline in bird's species and to know the causes of this decline at Taunsa Barrage wildlife sanctuary. Data were collected by line transect and direct census methods during wintering and breeding periods (2001 to 2002 and 2008 to 2011). The analysed results indicated an increasing trend in 157 species of birds during the observed period. A decreasing population trend in 14 species, however, was found. There was a 92% decline in White-backed Vulture (*Gyps bengalensis*), 60% in Greater Painted Snipe (*Rostratula benghalensis*), 57% Garganey (*Anas querquedula*), 50% in Long-legged Buzzard (*Buteo rufinus*) and Pallas's Fish Eagle (*Haliaeetus leucoryphus*) each, 39% in Little Cormorant (*Phalacrocorax niger*), 37% in Gadwall (*Anas strepera*), 30% in Black Drongo (*Dicrurus macrocercus*) and 13% in Red-crested Pochard (*Netta rufina*) population. The population of five species including, Marsh Harrier (*Circus aeruginosus*), Imperial Eagle (*Aquila heliaca*), Merlin (*Falco columbarius*), Common Buzzard (*Buteo buteo*) and Grey Heron (*Ardea cinerea*), however, remained unaffected during the observed period. The field surveys also indicated that the main causes of this decline in bird's population were habitat exploitation, fragmentation, hunting and deforestation in the sanctuary. This decline in bird's population may lead to successive

changes in the natural environment. It was concluded that conservation and management of declining bird's population is foremost to the interests of local community as well as to the environment. It is suggested, therefore, to take immediate steps for the protection of the sanctuary to conserve the declining bird's population.

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**ECOLOGY AND HUMAN-HIMALAYAN BROWN BEAR (*URSUS ARCTOS ISABELLINUS*) CONFLICTS IN MUSK DEER NATIONAL PARK, AZAD JAMMU AND KASHMIR**

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AWAN

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The Himalayan Brown Bear (*Ursus arctos isabellinus*) is considered as Critical Endangered in Pakistan. However, a small population of these bears still exists in northern Pakistan including Azad Jammu and Kashmir (AJK). A study was conducted to explore the ecology (with reference to Human-Brown bear conflicts) of Himalayan Brown bear in Musk Deer National Park (MDNP), AJK during April 2011 to September 2012. MDNP, covering 528.16 km<sup>2</sup> area, is located in the extreme north of AJK (upper Neelum Valley) on the both banks of the River Neelum at a distance of about 155 km from Muzaffarabad. A total of 17 transect surveys were carried out to collect the data on current distribution and population status, habitat and food preferences, and the patterns and extents of Human-Himalayan Brown bear conflicts in the study area. Besides, transect surveys, questionnaires surveys were also carried out (comprising 150 affected respondents/families) to collect the information about the human-bear conflicts and to evaluate economic losses, perceptions and tolerance of local people towards Brown bear. Study area was divided into three zones (Phulwai, Sardari and Loser) and the Brown bear signs were found in all zones. Based on direct and indirect signs of bear presence, a total population of about 11 individuals with a low population density (0.48/km<sup>2</sup>) was precisely estimated in the MDNP during the study period. Brown bear signs were found in all eight habitat types of the study area, however, Dry temperate coniferous forests (with 25% of all signs) were the most preferred habitat of these bears in the park followed by alpine meadows (24%) and sub-alpine scrub forests (20%), preferably along the flat terrain (38%), followed by undulating terrain (25%), gentle slope (25%), and steep slope (12%). The phytosociological analysis of vegetation of all study zones suggested about 38 plant species which was categorized on the basis of importance values (IV). Among these vegetations, *Abies pindrow* (average IV=30.13), *Viburnum grandiflorum* (average IV=24.76), and *Geranium wallichianum* (average IV=15.39) were found dominant among trees, shrubs and herbs in the bear habitat respectively. A sum total of about 27 plants species were found consumed by Brown bears which comprised about 77% of the total food composition along with animal matters (23%). Among plant and animal species, *Viburnum grandiflorum* and *Marmota caudata* were the most commonly

consumed species with about 18% and 8% contents respectively. Livestock killing by the brown bear was the major cause of the conflict as the human being commonly interfered into the bear habitats. During the last five years (2008-2012), a sum total of 63 livestock heads were killed by Brown bear which majorly comprises goats (n=36) and sheep (n=30). Most of the livestock killings were reported from inside the forest area (77%) in Alpine pastures (n=18) during the months of June (n=15) and July (n=13) at early night hours (1801-0000) (52%). Based on the market values of damaged livestock, a total of 1.414 million (Pakistani Rupees) economic loss of the local community was estimated with the highest during year 2012 (0.5300 million PKR). This economic loss leads towards the human-bear conflict by creating the negative perceptions about the bears in particular and other wildlife in general. Accordingly, about 96% of the respondents disliked the Brown bear and suggested to remove it from the area. Besides, there is no any conflict management strategy with the locals as well as with the government agencies. Based on findings of the studies it is concluded that although the Brown bear has its existence in the study area with very low population density, but the human interference and habitat overlapping by livestock creates conflicts between the human and bear which adversely affects the both species. The study suggests that a proper research based conflict management strategy should be developed and implemented with the integration of the local community to safeguard livestock killings and the conservation of the Critical Endangered Brown bears in the area.

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**ASSOCIATION OF SOME SELECTED SOIL INHABITING PREDATOR AND  
PREY SPECIES IN WHEAT-SUGARCANE AGRO-ECOSYSTEM OF  
FAISALABAD, PAKISTAN**

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Biodiversity at all levels is in continuous threat due to unwise management of community structure developed for higher yield of agricultural products to feed rapidly increasing human population. No insect population exists as an isolated entity. Rather, at any location many populations of organisms interact to varying degrees in a community. Many natural enemies of insects are polyphagous and have different relationship with various available preys. In agro-ecosystems predator-prey interactions are of great importance and insects as largest group play an important role to manage crop pests. Theoretically, these interactions are influenced by the structure and dynamics of an agro-ecosystem. Polynomial regression was applied by using Microsoft office excel 2007. The predator-prey interactions were determined on the basis of numerical superiority of a predator and its prey in a particular field. Analysis of the variety of predator and preys (which in most of the cases were also the pests on wheat) showed that *Formica spp.*,

*Camponotus* spp., *Solenopsis invicta*, *Oxychillus alliarius*, *Dolichoderus taschenbergi*, and *Clubiona obesa* were the dominant predators while *Armadilidium vulgare*, *Megomphix hemphilli*, *Armadilidium nasatum* and *Pangaeus bilineatus* were dominant preys in order of their abundance in the wheat fields while in sugarcane, *Formica* spp., *Solenopsis invic*, *Camponotus pennsylvanicus*, *Hippasa partita*, *Formica sanguinea*, and *Formica exsectoides*, were the dominant predators, while *Trachelipus rathkei*, *Hawaiiia minuscule*, *Pangaeus bilineatus*, *Biomphalaria havanensis*, *Planorbis merguiensis*, *Tritomegas sexmaculatus*, *Planorbis nanus*, *Gonocephalum stocklieni*, and *Pentodon idiota* were dominant preys. The data was analyzed for prey predator association by selecting dependant (predators) and independent (preys) variables in order to determine the optimum relationship by  $R^2$ -value. Polynomial regression analysis in wheat revealed that *A. vulgare* was the preferred prey of *Formica* spp. ( $R^2 = 0.955$ ) and *C. obesa* ( $R^2 = 0.839$ ). *M. hemphilli* was the preferred prey of *C. obesa* ( $R^2 = 0.972$ ) and *O. alliarius* ( $R^2 = 0.943$ ). While *P. bilineatus* was the most preferred prey of *D. taschenbergi* ( $R^2 = 0.857$ ). While in sugarcane Maximum association was showed by *S. invicta* and *T. rathkei* ( $R^2 = 0.988$ ). Similarly *F. exsectoides* showed significant association with *P. idiota* ( $R^2 = 0.942$ ) and *H. minuscule* ( $R^2 = 0.923$ ), and *H. partita* with *T. rathkei* ( $R^2 = 0.914$ ). *F. sanguinea* showed a significant association with *P. idiota* ( $R^2 = 0.884$ ) and *H. minuscule* ( $R^2 = 0.884$ ) whereas *Formica* spp. was associated with *T. rathkei* ( $R^2 = 0.843$ ), *S. invicta* with *P.* and *idiota* ( $R^2 = 0.842$ ) with *T. rathkei* ( $R^2 = 0.836$ ). Such type of studies is very important for designing and implementation of IPM techniques.

#### STATUS OF CAPE HARE (*LEPUS CAPENSIS* LINNAEUS, 1758) IN DISTRICT SUDHNOTI INCLUDING JUNJHAL HILL GAME RESERVE, AZAD JAMMU AND KASHMIR (PAKISTAN)

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Study was conducted to assess the current population status, distribution and habitat utilization of Cape Hare (*Lepus capensis*) in District Sudhnoti including Junjhal Hill game reserve, Azad Jammu and Kashmir (AJK), from March to December, 2012. For this purpose, direct (physical observation through transect method) and indirect methods (information through discussion) were used to collect information. The study area was divided into four main zones (Pallandri, Trarkhel, Mang and Bloch) and thirty nine sub-localities. The study was conducted at dawn and dusk while day time was utilized for taking indirect data. A total estimated population of about 196 animals was estimated at 39 different sub-localities of the study area. Among these localities, the highest population density (2.66 animals/km<sup>2</sup>) was found at Junjhal Hill Game Reserve in Zone Pallandri followed by Trarkhel (2.07 animals/km<sup>2</sup>), Bloch (1.62 animals/km<sup>2</sup>) and Mang (1.56 animals/km<sup>2</sup>). The overall population density recorded was 1.95 animals/km<sup>2</sup>

with the highest density index recorded at localities Junjhal Hill and Oora (3 animals/km<sup>2</sup>) in Pallandri Zone. The habitat of cape hare was quite rocky grassland and small cultivated areas with shrubs cover between 900-2200m elevation above sea level with dominant vegetation as *Pinus roxburghii*, *Pinus wallichiana*, *Acacia modesta*, *Olea ferruginea*, neem *Melia azadirachta*, *Ficus palmata*, *Ficus carica*, *Pyrus pashia*, *Ziziphus jujuba*, *Mallotus philippensis*, *Zanthoxylum alatum*, and *Melia azedarach*. Although the cape hare are least concern globally, however, habitat destruction, hunting, poaching, over grazing by livestock and disturbance by human activities were the major threats to the conservation of cape hare in the study area.

### **HABITAT CHARACTERISTICS AND ASSOCIATED REPTILIAN FAUNA IN CROPLANDS OF CHAKWAL, PAKISTAN**

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The study on habitat characteristics and associated reptilian fauna in croplands of Chakwal, Pothwar plateau, Pakistan was carried out from March 2013 to September 2013. Five types of crop fields at eight locations were selected to record reptile species diversity by visual encounter surveys and pitfall traps. The species occurring in each habitat/site was compared using hierarchical richness index (HRI). In total 11 reptile species were recorded including 6 lizards viz. *Calotes versicolor versicolor*, *Eublepharis macularius*, *Ophisops jerdonii*, *Eutropis dissimilis*, *Saara hardwickii* and *Varanus bengalensis* while the other five reptiles were snakes including *Ramphotyphlops braminus*, *Platyceph ventromaculatus*, *Ptyas mucosus mucosus*, *Bungarus caeruleus* and *Echis carinatus*. The Kruskal-Wallis test revealed that the means of the total number of individuals recorded from different sites differed non-significantly ( $P < 0.05$ ). A total of 177 specimens were recorded with *O. jerdonii* as the most abundant species with 62 individuals. *E. macularius* were rated as the least abundant species. Maximum number of individuals (53) was recorded from maize crop in Chumbi Surla wildlife sanctuary which constituted 30% of the all recorded individuals. Minimum number of individuals (8) was recorded from millet crop (Pir Ochri), which made up only 5% of the recorded individuals with the least HRI of 15. Results obtained from both Agglomerative Hierarchical Clustering and Canonical Correspondence Analysis indicated a strong association (both Positive and negative) of habitat variables viz. soil, vegetation, water resources, weather, human disturbance and predators with the abundance of reptile species. The study provides insights into the ecological role of reptiles in agro ecosystems of Pothwar area that could be used for conservation of this reptilian fauna.

**MICROHABITAT ASSOCIATION OF ANURANS INHABITING RAWALPINDI-ISLAMABAD AREA**

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The present paper deals with association of anuran species with microhabitat in Rawalpindi and Islamabad, Pakistan from September, 2012 to August, 2013. As many as 500 individuals of six anurans belonging to three families (Bufonidae, Dicroglossidae and Microhylidae) were recorded with a population density 0.185 ha<sup>-1</sup>. It was concluded that water quality and substrate (gravel size) had some levels of association with anuran abundance. Two anurans viz. *Limnonectes limnocharis* and *Duttaphrynus melanostictus* were associated with hydrophytes along the wetland margins while *Duttaphrynus melanostictus* with small gravel size. *Euphlyctis cyanophlyctis* was recorded from shallow water. Whereas *Duttaphrynus melanostictus*, *Bufo stomaticus*, *Hoplobatrachus tigerinus* and *Limnonectes limnocharis* were found at some distance from wetland margin and hop in the direction of water when approached. *Duttaphrynus melanostictus* preferred microhabitats with medium to low pH, low total dissolved solids, low electric conductivity, medium to high dissolved oxygen, small gravel size and high abundance of shrubs and hydrophytes while *Bufo stomaticus*, *Hoplobatrachus tigerinus* and *Limnonectes limnocharis* preferred a wide range of microhabitat settings. The recorded anuran species except *Microhyla ornata* are believed to be tolerant to disturbance due to traffic and construction activities.

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**REPRODUCTIVE PHYSIOLOGY OF FEMALE SMALL INDIAN MONGOOSE  
(HERPESTES JAVANICUS) INHABITING POTHWAR PLATEAU**

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Small Indian mongoose (*Herpestes javanicus*) plays an important role in the agro-ecosystem as it acts an important biological control species for rodents, snakes and many insects. It is well adapted to live near human habitation. The current study investigated its female reproductive physiology in the Pothwar Plateau. The females of the species were live trapped from their potential habitat of the study area from June 2012 to June 2013, and sacrificed to obtain blood and ovary tissue samples. The blood was centrifuged at 3500 rpm to obtain Plasma for estimation of hormones including estradiol, progesterone, FSH and LH, by using hormonal ELISA kits while ovary tissue samples were processed for standard histological procedure to cut and stain 5-7 um thick light microscopic sections. Results showed that the breeding season of small Indian mongoose in the study

area extends from late February till September whereas the non-breeding season of the species occurs from October till mid-February. During breeding season (March-September), estradiol, progesterone, FSH and LH concentrations were found elevated compared to non-breeding season (October-February). Average estradiol concentration during non-breeding season was  $168.36 \pm 36.8$  pg/ml which increased to  $445.1 \pm 143.4$  pg/ml during breeding season. Similarly, average progesterone concentration during non-breeding season was  $4.92 \pm 1.18$  ng/ml while during breeding season it elevated to  $8.44 \pm 2.09$  ng/ml. Average FSH concentration during breeding and non-breeding seasons were  $1.35 \pm 0.08$  mIU/ml and  $1.07 \pm 0.03$  mIU/ml, respectively whereas average LH concentrations were found to be  $9.76 \pm 1.7$  mIU/ml and  $14.21 \pm 3.6$  mIU/ml, respectively for non-breeding and breeding seasons. Two LH peaks were observed; one during March 2013, and the other during September 2012, indicating ovulation during these months. The corpora lutea were seen as prominent structures in the ovary during gestation period while during lactation phase of the species no corpora lutea or ripe follicles were observed. During non-breeding season ovaries depict estrus cycle and main structures observed inside were primary and secondary follicles, and graafian follicles.

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#### **COLLECTION, TRANSPORTATION, PROCESSING AND MARKETING OF RAZOR CLAM AND ITS ECONOMIC POTENTIAL**

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Razor Clam's trading was started in the year 2005 in Pakistan. The seafood exporters of country are largely involved in its collection and marketing as it is having tremendous export demand. About 84 frozen seafood exporter are present in our country. About 3000 Razor fishermen are present in the Sindh Province; whereas about 500 fishermen are active in its collection in the province of Balochistan. While about 20 Razor manufacturers/processors are present in Sindh, who are handling over the Live Razors to exporters at the Karachi, Air Port. However, in Balochistan 2-5 Razor manufacturer/processors are present. There is a very high profit margin in Razor Clam trading; therefore "profit margin" plays a vital role in the effort of Razor Collector. It is estimated that as a result of such effort Razor Clam become an endangered species in Pakistan. Some of its habitat are already destroyed due to pollution and other development activities. Due to these reasons, it is become necessary to work on "Marketing, Collection and processing of Razor Clam and current Economic potential" in Pakistan. Razor Clam is having no local marketing potential, because Pakistani Muslims do not take it as a food. Earlier it was exported to Malaysia. In Malaysia more than 40% population is Chinese, they were end user of Razors. Currently, our country is exporting it to China and Hong Kong. In this study, the authors are recording its current and past marketing status.

The complete collection process is noted in this paper. The losses during collection are 15%, it is proposed that how we can reduce these losses. Its process is entirely different as compare to the other seafood. Number of time it is put down into the water that is having temperature of 15 degree centigrade. Current and past history of processing is also recorded.



## 2. FRESHWATER BIOLOGY AND FISHERIES

### DETERMINATION OF GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN *LABEO ROHITA* FED WITH CANOLA MEAL BASED DIET SUPPLEMENTED WITH PHYTASE

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The study was designed to evaluate the effect of pyrase supplementation in acidified canola meal based diet in terms of nutrient digestibility and growth performance of *Labeo rohita* fingerlings. The six canola meal based diets were formulated by supplemented 0 and 500 FTU/Kg levels of phytase and 0, 1.5, 3% of citric acid. Chromic oxide was incorporated as an inert marker. The experimental diets were fed at 2% of net body weight. The experiment was run for six month. The higher weight gain of *Labeo rohita* fingerlings was recorded on diet having 500 FTU/Kg and 3% citric acid levels. The diets and pooled fecal material were analyzed for proximate analysis. Results indicated that canola meal based diet supplemented with 500 FTU/Kg and 3% citric acid significantly improved the nutrient digestibility (%) of dry matter ( $24.15 \pm 0.346$ ), crude protein ( $69.74 \pm 0.52$ ), crude fat ( $72.95 \pm 0.735$ ) and gross energy ( $64.95 \pm 1.49$ ).

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### EFFECT OF ZN + PB + NI MIXTURE ON THE GROWTH PERFORMANCE OF *CATLA CATLA* AND *LABEO ROHITA*

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An experiment was conducted to investigate the effect of sub-lethal concentration of metal mixture (Zn+Pb+Ni) on the growth performance of *Catla catla* and *Labeo rohita*. This experiment was conducted at constant water temperature (30°C), pH (7.25) and total hardness (225mgL<sup>-1</sup>). The duration of growth trial was of 90-day during which all the growth parameters viz. average increase in weight, feed intake, feed conversion efficiency and condition factor were monitored on weekly basis. Both control fish species performed significantly better than the metals mixture exposed fish species in terms of all growth parameters. Feed intakes were significantly better in the control fish while the treated fish fed below their intake capacity. Feed conversion efficiency and condition factor of fish were interrelated and the effects of chronic exposure of metal mixture on

the two parameters were significantly pronounced. The feed intakes of treated fish species were correlated positively and significantly with fish weight escalations, showing significant dependence of weight increments on feed intake by the fish. Condition factors of both treated and control *Catla catla* and *Labeo rohita* were directly and significantly dependant upon the trends of fish to gain weight.

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#### **NILE TILAPIA *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) CULTURE AT FISH HATCHERY, CHILYA, THATTA**

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The present study was conducted to evaluate rearing and culture of Nile tilapia (*Oreochromis niloticus*) for five months from May to September in cemented tanks (15 x 6 x 3 ft) at fish hatchery Chilya Thatta, Sindh. Nile tilapia fry (0.77 g weight and 0.78 cm length) were procured live from Thailand and stocked in cemented cisterns. After acclimatization the fry were fed with formulated diet containing 30% gross protein level twice a day at the rate of 5% body weight. The diet consists of fish meal, mustered oil cake, rice bran, wheat bran, rice protein, wheat flour, salt and vitamin premix. The water quality parameters were recorded monthly basis and ranged between temperature 27-35°C, dissolved oxygen 5.2-6.5 mg/lit, pH 6.7-7.2, Ammonia 0.5 mg/lit and hardness 70-185 ppm were found within the suitable ranges for fish culture. The results showed that the growth of fry was higher after 30 days of rearing period. Finally at the end of experiment fish attained (241 g weight and 25 cm length) in 150 day experiment.

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#### **EFFECT OF FEEDING FREQUENCY ON GROWTH PERFORMANCE, FEED UTILIZATION AND BODY COMPOSITION OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L.) CULTURED IN LOW SALINITY WATER**

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Feeding frequency is well known for the reduction of aquaculture production cost and for preventing water quality deterioration as the result of excess feeding. The

influence of feeding frequency was studied in the present study, when juvenile Nile tilapia *Oreochromis niloticus* (initial body weight 1.0 g) were maintained in rearing tanks (35 liters each, water temperature 29°C, salinity 14‰, pH 7.7, dissolve oxygen 5.1 ml/l and ammonia never exceeded 0.1 ml/l) and were fed diet of 35% protein for 42 days. Fish were fed at four frequencies: two, three, four and five times a day. Each feeding frequency was assigned to three tanks of fish with 10 fish per tank. Results showed that significantly higher weight gain, specific growth rate, feed conversion and protein efficiency ratio were observed at feeding frequency of four to five times daily. Moisture, protein and ash contents of whole body were not affected by feeding frequency. Lipid content of fish fed four and five times daily was significantly higher than that of the fish fed one and two times daily. The condition factor remained consistent at all feeding frequencies and survival was 100% throughout the experiment. These results suggest that under similar culture conditions, the optimum feeding frequency of juvenile Nile tilapia (from initial body weight of 1.0 g to 5.8 g) is four times daily.

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**EFFECT OF *SPHINGOMONAS* SP. ON SURVIVAL AND BIOCHEMICAL CONSTITUENTS OF *LABEO ROHITA* FINGERLINGS TO *V. ANGUILLARUM*.**

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This study was attempted to investigate the effect of *Sphingomonas* sp. AsCh-P3 as probiotics on survival and biochemical constituents of *Labeo rohita* fingerlings when challenged with *V. anguillarum* pathogen. Probiotic isolate having antibacterial potential against pathogen was sprayed on formulated fish feed. Positive control-C2 as well as experimental groups were challenged with *V. anguillarum* intraperitoneally, excepting the negative control-C1. Both control fishes were fed with sterilized feed. Probiotics treated feed was incorporated to experimental fishes for 45 days in different groups; G1 (soon after challenge for 30 days), G2 (15 days prior and 30 days after challenge) G3 (15 days prior to the challenge). Muscle tissues were analyzed biochemically. The survival rate was 60% in C2, 66% in G1, 83% in G2 and 76% in G3. Fishes subjected to *V. anguillarum* (C2) showed significant decrease in total Protein, Lipids, carbohydrates and DNA. The incorporation of probiotic isolate improved the protein, lipids, DNA in G2 and G3 groups while RNA contents differed significantly from control fishes in G1 group. A significant decrease in total carbohydrates was evident in all the experimental groups. On the basis of survival data and body profile, *Sphingomonas* sp. AsCh-P3 could be used successfully as probiotic bacterium against the pathogen in aquaculture.

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**DETERMINATION OF LEVELS OF HISTAMINE BY LIQUID  
CHROMATOGRAPHY-MASS SPECTROMETERY (LC-MS/MS) IN VARIOUS  
FISH SPECIES AVAILABLE IN LOCAL MARKET**

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Twenty five apparently fresh and properly ice samples of some inland Marine fish species were randomly purchased from five major fish market Punjab. The fishes transported to the lab in the ice boxes and temperature kept below -1°C. The samples were examined for appearance, glazing, texture, odour, presence of black spot and determination of levels of histamine by LCMS/MS. All the physical parameters were in normal range and there is no histamine present in any one of the samples. In conclusion, significant decomposition and histamine formation can be avoided by good fish handling practices including icing or rapid immersion of the catch in chilled water followed by uninterrupted frozen storage.

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**EFFECT OF COBALT SUPPLEMENTED DIETS ON ITS BIOACCUMULATION  
AND GROWTH OF *CYPRINUS CARPIO***

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Cobalt (Co) is an essential mineral required in trace quantity in fish diet. The present study was conducted to determine optimum level of Co which could be used as supplement in the diet to enhance the growth of *Cyprinus carpio* without constituting metal burden to the fish and to work out relationship between accumulation of Co in fish tissues and diet. Two separate trials were done with fingerlings of common carp, *Cyprinus carpio*: (i) growth and (ii) digestibility trial, with four different dietary levels of cobalt (T<sub>1</sub>, 0.0%; T<sub>2</sub>, 0.1%; T<sub>3</sub>, 1.0% and T<sub>4</sub>, 1.5%). Fingerlings of *Cyprinus carpio* were fed with cobalt-supplemented diet (CSD) with different dietary levels showed significantly higher growth than the control diet. This was obvious from the results of weight gain in treatment having Co 1.5% in diet (95.17% ± 0.62) as compared to control group (49.96% ± 1.14), feed conversion ratio in treatment having Co 0.1% in diet (2.46 ± 0.02) as compared to control group (2.81 ± 0.01), and specific growth rate in treatment having Co 1.5% in diet (0.537% ± 0.012) as compared to control group (0.307 ± 0.08). Accumulation of Cobalt increased in all tissues as compared with the respective initial values. Results of one-way ANOVA, using Co in liver, kidney, gill and gut as dependent

variables, revealed that there was significant variation of Co accumulation in all tissues between the dietary groups. Co accumulation significantly increased in all tissues (LSD;  $P < 0.01$ ) of fish fed 1.5% CSD ( $T_4$ ) as compared with other dietary groups ( $T_1$ ,  $T_2$ , and  $T_3$ ), except nearly similar values of Co in liver between  $T_3$  ( $17.58 \pm 1.13 \mu\text{g.g}^{-1}$ ) and  $T_4$  ( $20.05 \pm 1.03 \mu\text{g.g}^{-1}$ ) diets ( $P < 0.01$ ). Co level in all tissues of fish fed  $T_1$  ( $3.26 \pm 0.03 \mu\text{g.g}^{-1}$ ) to  $T_2$  ( $8.84 \pm 0.25 \mu\text{g.g}^{-1}$ ) diets showed similarity ( $P < 0.01$ ). Maximum accumulation of Co in fish fed 1.5% CSD ( $T_4$ ) was found in gut ( $97.72 \pm 0.69 \mu\text{g.g}^{-1}$ ) followed by kidney ( $37.06 \pm 0.98 \mu\text{g.g}^{-1}$ ), gill ( $27.59 \pm 0.75 \mu\text{g.g}^{-1}$ ) and liver ( $20.05 \pm 1.03 \mu\text{g.g}^{-1}$ ).

### ISOLATION AND IDENTIFICATION OF SOME PATHOGENIC BACTERIA FROM SELECTED FISH SPECIES OF RIVER RAVI

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The objectives of this study were to access the pathogenic bacteria in available fish species from three sites ie Siphon, Saggian and Sharkpur of River Ravi. Fish samples from each site were collected and analysed for five groups of pathogenic bacteria including Total Plate Count (TPC), Total Coliform Count (TCC), *Salmonella* species, *Vibrio cholera* and *Staphylococcus aureus* in *Cirrhinus mrigala*, *Catla catla*, *Labeo rohita*, *Seprata Seenghala*, *Rita rita*, *Wallago attu* and *Channa punctatus*. The results showed that highest Total Plate Count ( $2.31 \times 10^7$  cfu/g) in *Wallago attu* collected from Sharkpur and lowest ( $3.02 \times 10^4$  cfu/g) in *Seprata seenghala* collected from Ravi Saggian. Highest Total Coliform Count ( $< 210$  MPN/g) were observed in *Rita rita*, *Wallago attu* and *Seprata seenghala* respectively collected from Saggian, Sharkpur and Siphon and lowest ( $< 1$  MPN/g) in *Channa punctatus* collected from Siphon. Similarly the highest TFC ( $< 75$  MPN/g) in *Wallago attu* and *Rita rita* of Saggian and Shark pur and lowest ( $< 3$  MPN/g) in *Channa punctatus* collected from Ravi Siphon. *E.coli* was observed in three samples of each location. Pathogenic bacteria *Vibrio* and *Salmonella* species were detected only in two fish samples of each site collected from Saggian and Sharkpur. *Staphylococcus aureus* was found absent from all samples collected from three sites of the River Ravi. Two sites of River ravi viz., Sharkpur and Saggian were contaminated with high rate of TPC, TCC, TFC, *Salmonella* species and *Vibrio cholera* which indicates that waste water spilled in River ravi directly or indirectly.

**APPLYING CHEMICAL MEANS TO INCREASE DISSOLVED OXYGEN LEVEL DURING DRASTIC CONDITIONS IN WATER**

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Hydrogen peroxide is a strong oxidizing agent that is commonly used as a disinfectant, therapeutant, for improving water quality parameters and to increase dissolved oxygen for fish fauna in emergency situation where the mechanical aeration is not possible. The main focus of the present study was to evaluate the dissolved oxygen concentration with 6% hydrogen peroxide in relation to temperature factor. The result obtained showed that maximum oxygen release was 10 ppm at 12°C with the concentration of hydrogen peroxide 10ml/40L while when the same experiment was repeated at 31°C the maximum oxygen release was 7.5 ppm. With the increasing concentration of hydrogen peroxide *i.e.* 12ml/40L fish (*Ctenopharyngodon idella*  $\pm 2.8$ gms) mortality rate was 50% at 14ml/40L mortality was 75% and at 16ml/40L there was 100% mortality with the p-value  $\leq 0.05$  level of significance. During first hour of application dissolved oxygen increased upto the maximum then it was decreased to a minimum level (5.3 ppm) and that value persist for next 24 hrs. The use of hydrogen peroxide did not affect the physicochemical characteristics of water.

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**EFFECT OF ORGANIC AND INORGANIC FERTILIZERS ON GROWTH PERFORMANCE OF FIVE FISH SPECIES REARED UNDER POLYCULTURE SYSTEM**

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The present project was planned to determine the growth performance of five fish species *viz.* *Labeo rohita*, *Ctenopharyngodon idella*, *Cyprinus carpio*, *Catla catla* and *Cirrhina mrigala* under composite culture system. Three treatments with two levels of feeding strategy (F.S) each were used. In F.S #1 only organic and inorganic fertilizer were added where as in F.S #2 Supplementary feed was added along with fertilizers. The stocking density of all fish species was kept constant (30 each) except for *Cyprinus carpio*. *Cyprinus carpio* was stocked at the ratio of 40:30:20 in treatment # 1, 2 and 3 respectively. Water quality parameters were checked on daily basis to monitor the quality of water. In all the three treatments, maximum production were obtained in the treatment #1 (F.S. # 2) Where *Cyprinus carpio* was stocked maximum (40). Among all the five

species, *Ctenopharyngodon idella* showed maximum weight (g) gain where as *Labeo rohita* showed minimum gain in weight (g).

#### A PROCEDURE FOR ASSESSMENT OF FISH CONDITION ON THE BASIS OF LENGTH-WEIGHT RELATIONSHIP

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In this study a proposal for a factor is given to assess the condition in fish. The base here is not only total body weight and total length, but fish height is also considered. Here factor B in equation is represented as:  $B = M / (L^2 \times H)$  where M, H and L are fish body mass, fish height and length of fish, respectively. This was tested against the condition factor:  $K = M / L^3$  by using the data of *Notopterus* species. This was found that it provides a good prediction of fish weight from different body dimensions. From B it was assumed that body thickness is more correlated with length than fish height.

#### CHEMICAL COMPOSITION AND MINERAL PROFILE OF *LABEO ROHITA*, *WALLAGO ATTU* AND *CYPRINUS CARPIO* INHABITING RIVER INDUS IN MIANWALI DISTRICT

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The chemical composition and mineral profile of fish meat reflect its nutritional value and physical condition; it fluctuates extensively from species to species and within the same species in different weight categories depending on feeding habits and seasonal variations. This study assessed the chemical composition and mineral profile of three commercially important and widely consumed fish species namely *Cyprinus carpio*, *Labeo rohita* and *Wallago attu* inhabiting the River Indus in Mianwali District. The study followed a 3 x 3 factorial arrangement by involving 3 fish species, each with 3 weight categories ( $W_1 \leq 1.0$ ,  $W_2 \leq 1.5$  and  $W_3 \leq 2.0$  Kg). Fishing was performed with the help of local fishermen. Thirty samples of each fish species were selected from three weight categories on the basis of their routine weights at which they are caught and sold in the study area. The chemical composition of fish muscles was assessed by following the standard methods of Association of Official Analytical Chemists (AOAC). Selected

minerals (Ca, K, Mg, Na, P, Cr, Fe, Mn, Ni, Zn, Hg and Pb) in fish muscles were analysed by a Varian Vista-MPX CCD simultaneous ICP-AES (Varian Inc, Australia) machine located at Newcastle University, UK. Mineral concentrations were expressed as mg kg<sup>-1</sup> wet weight. Minitab 16 software was used to test the main effects of fish species, weights and their interaction for each mineral at P<0.05. The mean mineral contents in fish muscles were also compared with the International permissible levels for food fish. It was investigated that crude protein content in *Labeo rohita* was 74.92%, *Cyprinus carpio* was 75.25% and *Wallago attu* was 79.60%. Fat was reported as 18.67, 19.52 and 13.33 % in *Labeo rohita*, *Cyprinus carpio* and *Wallago attu*, respectively. While ash and total carbohydrates were reported as 4.95, 4.67 and 4.46 % and 1.46, 0.56 and 2.60 % in *Labeo rohita*, *Cyprinus carpio* and *Wallago attu*, respectively. Mineral contents in fish species were within the limits of international standards except Mn, Cr, Hg and Pb. The concentration of Pb in *Wallago attu*; Mn and Cr in all three fish species and Hg in *Cyprinus carpio* and *Labeo rohita* was found to be higher than the WHO/FEPA allowable limit for food fish. Cadmium and copper were not detected in any of the fish species analysed. Smaller fishes were high in Protein contents and low in fats and minerals, so these should be preferred by the consumers. Presence of heavy metals like Mn, Cr and Hg is a cause of concern as the consumption of these fish may cause metal related disorders in the consumers. This study has shown that Indus fish species are good source of nutrients but it's time to take preventive measure to reduce the bio magnification of heavy metals in fish.

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#### LENGTH-WEIGHT RELATIONSHIPS AND CONDITION FACTOR ANALYSIS OF FRESHWATER FISH SPECIES FROM THE RIVER INDUS IN MIANWALI DISTRICT

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This study assessed the effects of pollutants on Length-Weight relationships (LWR) and condition factor of selected freshwater fish species namely, *Labeo rohita*, *Cyprinus carpio* and *Channa marulius* sampled from the River Indus in Mianwali District. This study involved three locations around the River Indus in Mianwali District. Kukranwala (S1) site was designated as the reference site or low polluted site because of relatively less human activities in its surrounding areas. The other two sites called Shahbaz Khel (S2) and Ballo Khel (S3) were designated as medium and heavy polluted sites, respectively. Replicated water samples were collected from the three sites and were analysed for different physicochemical parameters *i.e.*, water temperature, pH, electrical conductivity, magnesium, calcium, total dissolved solids and total hardness. For the determination of weight-length relationships and wellbeing of Indus fish species, 360 fish



samples were collected from commercial fish sale points by involving 120 samples of each fish species from the neighbouring areas of selected locations. The morphometric parameters included in this study were total length, standard length, fork length and body weight. During this study, temperature fluctuated non-significantly ( $P>0.05$ ) while pH, total hardness, total dissolved solids, calcium and magnesium contents fluctuated significantly among reference and polluted sites ( $P<0.01$ ). Equations for LWR were computed as  $W=0.745 L^{1.517}$ ,  $W=1.270 L^{1.077}$ ,  $W=2.015 L^{0.854}$  for *Cyprinus carpio*, *Channa marulius* and *Labeo rohita*, respectively suggesting negative allometric growth in all the species which may be attributed to the stress exerted by river contamination through heavy metals, pesticides and other effluents. Mean condition factor computed for *Cyprinus carpio*, *Channa marulius* and *Labeo rohita* was 2.705, 2.13 and 3.38, respectively indicating that *Labeo rohita* exhibited better health condition as compared to other species. Further comprehensive investigative approach is required to ascertain the influence of pollutants in River Indus water and their impact on aquatic fauna.

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#### FACTORS RESPONSIBLE FOR EMPLOYEE TURNOVER IN THE FISHERIES INDUSTRY OF KARACHI

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The growth of fishery industry is not increasing rapidly. The main reason is uncertainty of fish catch. Due to such uncertainty the daily income of employees in this industry is decreasing, and in turn, increasing employee turnover in the industry. The skilled labours are not ready to join a new boat/firm without six months advance salary. Due to such an alarming shortage of labour, the author explores five "Work Factors" which might be responsible for employee turnover. The first factor is time spent on work, second compensation/wage/share, third work environment or interest in work, fourth career progression and the fifth is over all job satisfaction. Each of these "Work Factors" is further divided into three more categories. This study examines the 500 employee responses and their preferred "Work Factor" from the targeted industrial population of 600 firms. The 16 different reasons for leaving the organization were organized into categories. These categories have similarities amongst them. Significant differences were found in the factors given for "intention of leaving". It is based on cadre, age, marital status, income and gender. Out of 5 "Work Factors", the first factor is proved to have no relationship with employee turnover. The second, third and fourth factors were identified to be the most important factor for employee turnover. Although "salary" was found to be the most important work factor for the employee's decision to stay or leave, other factors also contributed towards employee turnover. These factors further need to be explored and identified. We also observe that male respondents have shown a clear preference for "salary" in determination of employee turnover whereas; the female respondents have given equal preference to "salary", "learning opportunities" and "relationship with the

boss". Respondents belonging to the younger age bracket have shown inclination towards "salary" whereas, the respondents belonging to an older age bracket likely give consideration to other factors in deciding to leave or stay with the company. Married respondents favored "salary" over other factors, whereas, single respondents show a preference for "learning opportunities" over other factors. Junior management cadre preferred "salary" and "relationship with the boss", whereas, senior management cadre shows a preference for "salary" and "career progression". A total of eight salary brackets were made to check preferences for work factors. Respondents falling in lower salary brackets gave preference to "non monetary work factors", whereas, respondents possessing higher salaries preferred "salary" over other work factors.

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**DETERMINATION OF HEAVY METALS AND THE GROWTH  
PERFORMANCE OF *Oreochromis niloticus* REARED UNDER  
TREATED WASTEWATER DILUTIONS**

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To determine heavy metals and growth performance of *Oreochromis niloticus*, four concentrations of wastewater *i.e.* 25%, 50%, 75% and 100% were made and each aquarium was filled with respective concentration. One aquarium was filled with tap water to keep it as control. The test medium was renewed on weekly basis to maintain the desired wastewater dilution. Throughout the experimental period of 12 weeks fish were fed to satiation twice a day. Physico-chemical parameters viz. water temperature, pH, total hardness, dissolved oxygen were monitored on daily basis. However, water temperature (30°C) and pH (7) were kept constant throughout the study period. At the end of 12 week experiment, the growth of fish in terms of feed intake, feed conversion ratio, negative or positive increase in wet weights along with fork and total lengths were observed. At the end of the experiment, the fish from each aquarium was dissected and tissue samples were digested in aqua regia for the analysis of heavy metals under atomic absorption spectrophotometer. The data on different parameters of fish growth and physico-chemistry of water were subjected to statistical analysis through MSTAT and MICROSTAT packages of the computer. The results showed that the treated fish exhibited significantly lower weights and length gains than the control fish. Wastewater exposure to the fish resulted in increased oxygen consumption and higher ammonia excretion by the fish. Moreover the presence of certain waterborne and dietary metals in treated wastewater accumulated in fish body organs as follows: liver < kidney < gills < bones < skin < muscles < fins.

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**PREPARATION OF FISH PASTE AND FISH FLOUR FROM FRESHWATER  
SPECIES *LABEO ROHITA* AND *CATLA CATLA***

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Fish paste is considered to be one of the main food sources. While the fish flour is idealized to promote fish flavor in the confectionaries. Unfortunately, it haven't much importance in Pakistan. However, it is a best source of protein and work as intermediate product in the world of cooking and baking to make various recipes. Two freshwater fish species were taken named *Labeo rohita* and *Catla catla*. The meat were used to make fish paste and flour individually. Fish Paste were made with *Labeo rohita* and *Catla calta* were sensory evaluated. The both fishes were taken and meat portion were isolated to make paste by blending meat black peppercorn and salt separately while kept one as plain paste without any flavor. Both pastes were rich in total ash content and had a protein content exceeding 12%. The sensory evaluation results showed high preference of *Labeo rohita* paste mixed with pinches of black peppercorn. While on the other hand the flour were made by same two species by grinding the meat after oven dry at 80-90 C for 3-4 days and then treated with ordinary flour to enhance the flavor in the percentage of 5%, 10%, 15% and 20%. The Sensory evaluation results for the fish flour showed high preference for both fishes at 10%. The incorporation of fish flour at levels up to 10% does not alter the taste and appearance of bread.

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**LENGTH WEIGHT RELATIONSHIP, CONDITION AND FEEDING  
SELECTIVITY OF THREE CARANGID FISHES IN PAKISTAN**

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This study is based on the samples obtained from Karachi fish harbour from July to Dec 2013, we estimated length weight relationship, relative condition factor *Kn* and feeding habits in *Megalaspis cordyla*, *Scomberoides lysan* and *S. tol* of the family carangidae. The length weight relationship was estimated with general parabolic equation  $W = aL^b$ . From value of  $> 0.9$  of the regression coefficient, there is strong linearity between the parameters and slightly negative allometric relationship between length and weight is obvious. Relative condition factor *Kn* calculated as *Megalaspis cordyla* *Kn* = 0.740min – 1.380max, *Scomberoides lysan* *Kn* = 0.896 – 1.146 and *S. tol* *Kn* = 0.540 – 1.141. Feeding selectivity of the each individual was examined and estimated as % of numerical composition, % of gravimetric composition and % of frequency of occurrence.

The fullness of the stomach recorded as empty, 25%, 50%, 75%, and 100% whereas gut contents of the three species varied in number, weight, and their frequency of occurrence from species to species. Present data of important species of the family carangidae would greatly increase understating of the stock structure and fishery management.

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**EFFECT OF ACIDIFICATION IN A PHYTASE SPRAYED SUNFLOWER MEAL BASED DIET ON GROWTH AND DIETARY NUTRIENT DIGESTIBILITY PERFORMANCES OF *LABEO ROHITA* JUVENILES**

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An experiment was conducted to investigate the effect of citric acid supplementation on a diet consisting of sunflower meal as major protein source in *L. rohita* juveniles. Three experimental diets were made by supplementing phytase at constant level (750 FTU/kg) while citric acid was added at the level of 0%, 1% and 2% in the basal diet resulting in the formation of three experimental diets designated as SFM<sub>1</sub>, SFM<sub>2</sub> and SFM<sub>3</sub>. Chromic oxide (1%) was added to all experimental diets to measure the dietary nutrient digestibility. Growth and digestibility performances of juveniles showed significant ( $p < 0.05$ ) responses against diet acidification. Maximally improved ( $p < 0.05$ ) weight gain and feed conversion ratio were observed in SFM<sub>3</sub> diet supplemented with 2% citric acid. Likewise, maximum apparent dietary nutrients digestibility was also recorded in SFM<sub>3</sub> diet. In conclusion, acidification of sunflower meal based diet with citric acid improved the growth performance and nutrient digestibility in *L. rohita* juveniles.

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**QUALITY CHANGES AND SHELF LIFE OF CHILLED AND SUPER CHILLED MORAKHI (*CIRRHINUS MRIGALA*)**

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Fish and fish products are now transported at international, national and regional level and hence the freshness or quality of these products becoming more and more

important. As a healthy alternative to other animal protein, they are on the increasing demand all over the world. There is need to utilize low value fish by using preservative techniques and assuring quality measures. A study on the shelf life of Mori (*Cirrhinus mrigala*) 280±300g in chilling and super chilling temperature was carried out. The storage life of the fish was 20 days in refrigerating temperature (0± -25°C) and (0± -4°C), respectively. TVB-N values continuously increased with the lapse of the storage period. TVB-N value increased from 4.2mg/100g to an acceptable value of 22.53 mg/100g in 15 days and finally to a rejection value of 38.75 mg/100g at the end of 21days storage period, TVB-N results showed acceptability for 7 days of chilled and 24 days of super chilled storage. Organoleptic acceptability was also 5-7days in (0 ±25°C) and about 18-20 days at (0± -4°C) temperatures. Results of bacterial load showed that the samples were in highly acceptable condition, not exceeding 106cfu/g for about 20 days chilled and 89cfu/g at super chilled temperature. Colour change testing results were acceptable at both temperatures whitish pink at (0±25°C) and dark pink at (0± -4°C). pH Of the fish before preservation was 6.9 after storage it was 4.6 and 5.5 respectively. Fish meat swelling capacity (SW) that record after storage was acceptable in storing temperature (0± -4°C) 42.4% while increased in storing temperature (0-25°C) 57.1%. Extract release volume (ERV) measured value was >25ml in super chilled (0± -4°C) and >20ml in chilled (0±25°C) temperature. Texture of super chilled (0± -4°C) was more acceptable in sensory evaluation than chilled (0±25°C).

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#### INFLUENCE OF FRESHNESS AND FROZEN STORAGE TEMPERATURE ON QUALITY OF THAWED FILLETS OF *PANGASIU PANGASIU*

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The quality of the fish deteriorate due to fluctuation of the temperature, too long storage times and use of raw fish material of an insufficient degree of freshness. These factors are known to result in a high number of bacteria causing amine odour and spoilage in fish. A simple two factor, two level factorial designs was used to evaluate the freshness and frozen storage temperature on the quality of the thawed fillet of *Pangasius Pangasius*. Four sub-batches of *Pangasius* fish fillets (A, B, C and D) were packed. These fillets were packed just after an hour from the market. A and B was weighed and placed in a plastic tray including with foil paper. Each tray containing one fish "fillets of approximately 250-300 g they were place in the chilled condition below 8°C to 5°C with maximum temperature of 0°C. *Pangasius* fillets used in batch C and D were wrapped with polyethylene and aluminum foil and stored aerobically in boxes at -5 to -15°C for a further 7 days before packaging in deep freezing atmosphere. The *Pangasius* fillets in the

four batches were frozen and chilled. After freezing, batches A and B were stored at -15 to -3°C and batches C and D were stored at chilled. After 6 week of frozen storage, the *Pangasius* fillets were thawed in water for 1 hour. Seven times during chill storage (after 0, 3, 5, 7, 11, 14 and 17 days) seven packs with *Pangasius* fillets from batch A and batch B and batch C and batch D were used for analysis. At each sampling time four packs of each batch were used for sensory evaluation. The results showed that fish preserved in frozen environment as compared to chilled seems to be a promising technique for long term preservation while quality maintain at chilled storage.

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#### **EFFECT OF ACETIC ACID AND ASCORBIC ACID ON THE PRESERVATION AND NUTRITIONAL VALUE OF *LABEO ROHITA***

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Nutritional value of fish decreases if its preservation is not up to mark. Acetic acid is a carboxylic acid known as Ethanoic acid ( $C_2H_4O_2$ ) it is an organic acid and it is beneficial for the preservation of fish meat. It increases the values and add vitamin in fish flesh. This study was conducted to evaluate the difference between acetic acid and ascorbic acid in the preservation of fish (*Labeo rohita*). For this purpose, short term and long term preservation was evaluated on this important fish specie. Short term preservation was based on 10 days while long term preservation was of 20 days. The amount of 1.0 M of acetic acid and ascorbic acid was used which have  $5.6 \pm 0.2$  pH. In long term preserved fish was kept in acids of 1.0M in refrigerator at 4°C for 20 days. After 10 days of trial fish was taken again and preserved in acetic and ascorbic acid for short term preservation. The results showed that the proximate composition of whole body of *Labeo rohita* which received the acetic acid have Crude Protein 75.58, Moisture 11.46, Ash 2.01, Fat 7.46) and initial reading are (crude protein 70.36, Moisture 9.13, Ash 2.55, and Fat 11.78.) while fish received ascorbic acid contained (crude protein 72.56, Moisture 10.23, Ash 3.25, Fat 9.21) and initial readings are (crude protein 71.95, Moisture 7.99, Ash 3.02, and Fat 12.02). No significant difference between initial readings of proximate analysis found. Microbial load of fish preservation with acetic acid was  $2.1 \times 10^6 \pm 1.0$  while in ascorbic acid was  $3.12 \times 10^6 \pm 0.9$ . In this study the preservation with acetic acid was more effective than ascorbic acids while microbial load is also less as compared to ascorbic acid. A nutritional value of acetic acid is more as compared to ascorbic acid and is more beneficent for taste point of view.

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**ROLE OF PHYTASE SUPPLEMENTATION IN IMPROVING GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF *CIRRHINUS MRIGALA* FINGERLINGS FED ON SOYBEAN MEAL-BASED DIET**

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Presence of phytic acid in plant ingredients reduces the bioavailability of protein and minerals to fish ultimately resulting in reduced fish growth. The present research work was conducted to examine the effects of phytase supplementation (0, 500, 1000, 1500 and 2000 FTU kg<sup>-1</sup>) to soybean meal diet on nutrients and mineral availability for *Cirrhinus mrigala* fingerlings. Chromic oxide was added as indigestible marker. *C. mrigala* fingerlings were stocked in V-shaped tanks in Fish Nutrition Lab particularly designed for the collection of feces. Triplicate tanks were used for all treatments. Feed was given to fingerlings at the rate of 5 % of live wet weight of fish. From each tank feces were collected twice daily. Collected feces were dried at 60°C and pooled for each treatment. Water quality parameters viz., DO, temperature and pH in each tank were monitored throughout the study period. The results showed that 1000 FTU kg<sup>-1</sup> level in soybean meal based test diets increased the bioavailability of nutrients and minerals to *C. mrigala* fingerlings which in turn resulted significant (P<0.05) increase in growth performance parameters. Therefore, phytase supplementation at 1000 FTU kg<sup>-1</sup> level in plant ingredient (soybean meal) based diets was suggested as optimum level of phytase supplementation to enhance the release of chelated nutrients and minerals. Use of phytase in diets reduces the nutrient and minerals discharge into aquatic environment resulting in less aquatic pollution. Supplementation of phytase also helped in developing cost-effective and eco-friendly fish feed.

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**EFFECT OF DIFFERENT LEVELS OF PROBIOTIC SUPPLEMENTS ON GROWTH INDICES AND BODY COMPOSITION OF JUVENILE *CYPRINUS CARPIO* AND *OREOCHROMIS NILOTICUS***

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The present project was planned to evaluate the use of a commercially available probiotic (Biogen) for *Cyprinus carpio* and *Oreochromis niloticus*. During the experiment, both species were equally distributed into two groups A and B. Group A was fed @ 4% of body weight and group B @ 6% of body weight daily. Each group was

further divided into five probiotic levels *i.e.* for group A; A<sub>0</sub>, A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> and A<sub>4</sub> and for group B; B<sub>0</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and B<sub>4</sub> corresponding to 0, 2, 4, 6 and 8% of probiotic level respectively. *C. carpio* produced maximum weight gain (10.20) and specific growth rate (1.046) in treatment A<sub>4</sub>, while *O. niloticus* produced maximum weight gain (9.73) and specific growth rate (2.774) in treatment B<sub>4</sub>. Both species produced maximum survival rate (100%) in probiotic treated groups. Lowest FCR (0.275, 0.227) was observed in B<sub>4</sub> and A<sub>3</sub> for *C. carpio* and *O. niloticus* respectively. There was no significant differences ( $p>0.05$ ) in proximate composition parameters except protein which increase significantly as the probiotic level increased. This study demonstrates that up to 6 and 8 % of probiotic (Biogen) can be substituted for fishmeal in a fishmeal-based diet for *C. carpio* and *O. niloticus* respectively without any side effects on growth and proximate composition.

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**PHYTASE SUPPLEMENTATION OF DRY BREAD MEAL BASED DIET  
ENHANCES THE GROWTH PERFORMANCE AND NUTRIENT  
DIGESTIBILITY IN *LABEO ROHITA* FINGERLINGS**

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The influence of top spraying of phytase on dry bread meal (DBM) based diet was studied in *Labeo rohita* in relation of growth and nutrient digestibility. Eight experimental diets including one reference diet and seven test diets were prepared. Test diets were comprised of 70% reference diet and 30% dry bread and designated as DBM<sub>1</sub>, DBM<sub>2</sub>, DBM<sub>3</sub>, DBM<sub>4</sub>, DBM<sub>5</sub>, DBM<sub>6</sub> and DBM<sub>7</sub> and were supplemented with phytase at the level of 0, 250, 500, 750, 1000, 1250 and 1500 FTU/kg respectively. Chromic oxide was added at 1% level of reference diet and used as inert marker. Improved ( $p<0.05$ ) growth performance and feed conversion ratio (FCR) were observed in test diet DBM<sub>4</sub>, supplemented with phytase at the level of 750 FTU/kg while these vary non-significantly from reference diet. Dry matter, crude protein, crude fat and gross energy also showed improved ( $p<0.05$ ) apparent digestibility coefficient (ADC) in DBM<sub>4</sub> diet compare to other test diets and reference diet. Results from present study indicate that Rohu digested the dietary nutrients better when DBM based diet was supplemented with phytase at 750 FTU/kg level resulting in improved growth performance.

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### **APPLICATION OF CITRIC ACID SUPPLEMENTATION IN FISH FEED**

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Fishes usually have low levels of acid secretion in the stomach as compared to mammals. Inclusion of organic acids in diet reduces the pH of gut in fish. It is well documented that lowering of pH increases the phytate hydrolysis, kills the pathogens, decreases the rate of gastric emptying and improves mineralization and nutrient absorption. Among the organic acids, citric acid due to its unique flavor and high buffering capacity has been extensively used for diet acidification. It has the great potential to replace fish meal (up to 70%) with plant protein sources. Being a strong chelator of calcium and phosphorus, citric acid enhances the phytate hydrolysis. It improves the bioavailability of minerals by solubilizing the bones and competing with other chelators. It also increases the endogenous and exogenous phytases efficiency by providing an optimum pH in gut. Besides, it acts as antimicrobial agent and stimulates feeding in fish. The major objective to review the applications of citric acid supplementations in fish feed is to highlight its role in improving growth performance, nutrient digestibility, minerals availability and phytase efficacy.

### **CONSUMER SATISFACTION FOR FISHERIES PRODUCT OF LARGE DEPARTMENTAL STORE VERSUS SMALL FISH SHOP IN PAKISTAN AND MALAYSIA**

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Fish consumption trend is very poor in Pakistan as well as in Malaysia. Alternate foods are increasing obesity and it is also a major cause of death in Malaysia. Doctors are promoting the use white meat. The white meats of boiler chicken are causing cancer in Asian countries. Hence, the fish meat is left as a source of save/cheap nutrition. Its consumption is low in both the countries due to non-fresh stock. The large departmental stores are now trying to sale fresh fisheries products. But the fish consumers are still uncertain/indifference about consumption of fish from large store or small shops. Therefore, in this study the consumer satisfaction level is tested. Data about price, quality, consumer income consumer taste, etc is taken on 400 questionnaires from both

counties. The results of survey were tested via indifference curve of consumer behavior theory. The theory was separately applied for both of the counties. It is found that consumer in the both counties is more satisfied with the large departmental stores. The budget line of Pakistani consumer is tangent to indifference curve no.3 *i.e.* ID 3. Where, in case of Malaysia it is tangent to ID 4. Its is concluded that the consumer is less satisfied with small shops due to low quality of fish, what is also a cause of low consumption of fisheries in both of the countries.

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### MARKET STRUCTURE OF FISHERIES INDUSTRIES OF SINDH

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The World is globalizing, but Pakistan's fish industry's speed is very slow in this regard. Apart from the rest of the hurdles in this process, the unexplored/non documented market structure is a major hurdle. This industry has eight major segments. These segments have 380 units for edible marine fisheries, 0 units for edible marine fish farming, 450 units for edible inland wild fisheries, 580 units for edible inland fish farming, 7 units for wild marine aquarium fishes, 0 units for farmed marine aquarium fishes, 59 units for wild aquarium inland fishes and 9 units for aquarium inland farmed fishes. In this study the market structure of these eight segments is documented. To explore the size of firms, the number of employees in different years is noted in a table. All these segments have different consumers, and these consumers do not interact with each other. Monthly sale of all the firms is produced in a table, along with the contracts of fish firms. We notice that the 15 firms are similar with respect to their average sale. We computed the Concentration Ratio by adding the yearly sale of all the units and dividing by the total by 16. The average CR16 is 0.05.

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### EFFECT OF FERTILIZERS AND SUPPLEMENTARY FEEDING ON WATER QUALITY AND PLANKTON PRODUCTIVITY IN FISH PONDS UNDER UNIFORM FISH STOCKING DENSITY

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Studies were conducted to investigate the effects of different fertilizers and supplementary feed on water quality parameters. Dissolved oxygen levels were higher in nutrient rich and plankton dense ponds. There were quite minor variations in water quality parameters among treatments and different seasons of the year. Values of alkalinity and hardness remained same irrespective of the type of input. Total solids were

much higher in those ponds with both types of fertilizers and supplementary feed. Differences were more prominent in the levels of plankton productivity which was always higher in nutrient dense ponds where all the proposed inputs were present. During the winter months this difference however totally mitigated indicating low metabolic and decomposition activities. So it can be concluded that water quality can be maintained within acceptable ranges if all the inputs are added in well measured and managed way. Haphazard and poorly managed activities always lead to deleterious effects.

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**ISLAMIC BANKING, COTTAGE MARINE FISHERIES INDUSTRY, INTEREST FREE LOAN DEMAND, AVERAGE MONTHLY INCOME AND UNSUCCESSFUL FISHING TRIPS WITH REFERENCE TO LOCAL FISHERMAN IN KARACHI**

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Cottage level fishermen are in poverty cycle since couple of decades in Karachi. They are unable to break this cycle without any small external financial support. Early studies report that it is due to poor income habit and poor health habit in them. But in this study, it is hypothesized that the boom of Islamic banking in Pakistan and Prime Minister Nawaz Sharif interest free loan scheme can broke the vicious poverty cycle of fisherman. In these connection 1000 surveys was done to fix the main problems of fisherman. It is found that the most of the cottage fisherman are 16 times going for fishing in a month. On average, they are having 6 unsuccessful fishing attempt and 2 sick family members in every month. What is consuming their 4 days earning. It is concluded that small interest free loans and health education can broke the vicious poverty cycle of our fisherman. In this study the loan practices of local Islamic banks is also criticized and proper recommendations are given.

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**BIOACCUMULATION OF METALS IN FISHES' SCALES – A RELIABLE ASSESSOR OF FOOD SECURITY**

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In this study, we collected three fish species (*Cirrhinus mrigala*, *Labeo rohita* and *Catla catla*) from four sites (siphon=A, shahdera=B, sunder=C and Balloki headworks=D) during two flow seasons, to analyzed the accumulation of cadmium (Cd),

chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) in fish scales. All the studied metals contents were significantly different ( $P < 0.001$ ) among the sampling sites and flow seasons. Site wise metals accumulation pattern was site C > D > B > A. The highest concentrations ( $\mu\text{g/g}$  dry weight) of Cd (0.29), Cr (4.64), Cu (8.85), Fe (65.66), Mn (5.14), Hg (2.91), Ni (3.18), Pb (5.14) and Zn (72.16) were recorded at site C. Among sampled fish species, *C. mrigala* showed highest potential of metals bioaccumulation than *L. rohita* and *C. catla*. All scales samples of fishes caught during low flow season showed significantly ( $P < 0.001$ ) higher concentration of metals than high flow seasons. The variations in metals contents in fishes scales are attributed to variations in heavy metals contents in the environment. The fishes scales thus can be used as a biomarker of aquatic pollution. An added advantage is that these can be used without sacrificing the animal.

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#### ICHTHYOFAUNAL DIVERSITY AND ASSESSMENT OF WATER QUALITY OF MAJOR STREAMS OF DISTRICT SHANGLA, KHYBER PAKHTUNKHWA, PAKISTAN

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The present study of the streams of, District Shangla, K.P., Pakistan, was conducted for six months, from March to Augusts 2013 to find out their ichthyofaunal diversity and water quality parameters. Fishes were collected by using different types of nets of various mesh sizes including cast nets and others local fishing devices. During the study period, 11 fish species were recorded from the streams of District Shangla. Family Cyprinidae was recorded to be the dominant fish family represented by 4 species viz. *Schizothorax plagiostomus*, *Schizothorax esiocinus*, *Orienus plagiostomus* and *Racoma labiata*. Family Namacheilidae was represented by 3 species namely *Schistura lepidocaulis*, *Schistura prashari* and *Schistura curtistigma*. Family Sisoridae was represented by three species *Glyptothorax punjabensis*, *Glyptothorax stocki* and *Glyptosternon maculatum*, whereas Family Mastacembelidae was represented by only one species, *Mastacembelus armatus*. During the study period, regular examination of the physicochemical parameters of the water was also taken. Their mean values were; temperature 16.75 °C, pH 6.68, electric conductivity 181.40  $\mu\text{S/cm}$ , alkalinity 202.28mg/L, total suspended solids 59.17 mg/L, total dissolved solids is 91.90mg/L, free carbon dioxide 4.61mg/L, total hardness 97.5mg/L, calcium hardness 61.45mg/L, magnesium hardness 36.83 mg/L, salinity is 0.03 mg/L and the mean velocity of water was recorded as 1.9m/s. This is the first study ever made in District Shangla which explored not only the fish fauna but also the water quality of the major streams of the area. It is concluded that the area is suitable for fish survival and efforts should be made

to utilize vast water resources of this area for poverty alleviation and socio-economic uplift.

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**EFFECTS OF VARYING RATIO OF *CYPRINUS CARPIO* ON GROWTH OF FISH SPECIES IN COMPOSITE CULTURE SYSTEM**

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A composite polyculture experiment was carried out to compare the growth performance of five fish species viz. *Labeo rohita*, *Ctenopharyngodon idella*, *Cyprinus carpio*, *Catla catla* and *Cirrhina mirgala*, under the influence of fertilization and supplementary feed in different combination was conducted in six earthen ponds having area of 0.17 acre with two replications for each. 3 treatments each with two levels of fertilization strategy were used. The stocking density of *Cyprinus carpio* varied in each treatment whereas stocking density of *Labeo rohita*, *Ctenopharyngodon idella*, *Catla catla* and *Cirrhina mirgala*, was kept constant. Physico chemical parameters were checked on daily basis. In all three treatments, maximum fish productivity was obtained in treatment#1, feeding strategy#2 (feed+fertilizer). Among all the five fish species *Ctenopharyngodon idella* showed maximum growth in terms of wet weight and length followed by that of *Labeo rohita*, *Catla catla*, *Cirrhina mirgala*, and *Cyprinus carpio*.

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**ENHANCING DIGESTIBILITY OF NUTRIENTS FOR *LABEO ROHITA* THROUGH SUPPLEMENTATION OF PHYTASE AND CITRIC ACID**

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The aim of the project was to enhance digestibility of nutrients for *Labeo rohita* through supplementation of phytase and citric acid. The experimental diets were supplemented with phytase (0, 750 and 1000 FTU kg<sup>-1</sup>) and citric acid (0, 1.5 and 3%). 1% chromic oxide (non digestible marker) was used to determine nutrient digestibility. Triplicate tanks were assigned to each experimental diet. The diets were fed at 2% live wet weight of fish. Fecal matter was collected daily for determining nutrient contents. Nutrient and mineral digestibility were determined by standard methods. Water quality parameters were controlled and kept constant throughout the experiment. Crude protein,

crude fats, dry matter and gross energy digestibility were significantly higher at 3% citric acid and 1000 FTUkg<sup>-1</sup> phytase level. Ca, Mg, P, Fe, and K digestibility was also highest at 3% citric acid and 1000 FTUkg<sup>-1</sup> while Na and Mn digestibility was highest at 3% citric acid and 750FTUkg<sup>-1</sup>. Zn digestibility was best at 1.5% citric acid and 0 FTUkg<sup>-1</sup> phytase. 1.5% citric acid and 1000 FTUkg<sup>-1</sup> was the optimum level for Cu digestibility. This research very clearly depicted that 3% citric acid and 1000 FTUkg<sup>-1</sup> is the best level for enhancing nutrient digestibility for *Labeo rohita*.

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**ICHTHYOFAUNAL DIVERSITY AND ASSESSMENT OF WATER QUALITY  
OF BARAN DAM, DISTRICT BANNU, KHYBER PAKHTUNKHWA,  
PAKISTAN**

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The present study of the Baran Dam, District Bannu, K.P., Pakistan was conducted from September to December, 2013 to explore its ichthyofaunal diversity and to assess its water quality. Fishes were collected with the help of professional fishermen by using different types of hooks and nets of various mesh size including cast nets, scoop nets, hand nets, fry nets, drag nets and drift gill nets. During the study period 28 fish species were recorded from the Baran Dam. Family Cyprinidae was recorded to be the dominated fish family represented by 17 species viz., *Aspidoparia morar*, *Barilius naseeri*, *Barilius modestus*, *Barilius vagra*, *Crossocheilus diplocheilus*, *Cyprinus carpio*, *Carassius auratus*, *Cirrhinus mrigala*, *Chela labuca*, *Hypophthalmichthys molitrix*, *Labeo boga*, *Labeo diplostomus*, *Labeo dyocheilus*, *Puntius ticto*, *Puntius sophore*, *Puntius conchoni* and *Salmostoma bacaila*. Family Bagridae was represented by 3 species viz. *Mystus cavaisus*, *Mystus tengra* and *Sperata sarwari*. Family Channidae was represented by 2 species namely *Channa punctata* and *Channa gachua*. Among less common fish families, Family Siluridae by was represented by *Ompok pabda*, family Sisoridae by *Glyptothorax naziri*, family Cichilidae by *Oreochromis niloticus*, family Chandidae by *Chanda nama*, family Mastacembelidae by *Mastacembelus armatus*, and family Notopteridae was represented by *Notopterus notopterus*. During the study period, regular monthly investigation of the physicochemical parameters of the water was also performed. The mean values of different parameters were; pH 7.11, electrical conductivity 490.75 µs/cm, alkalinity 82.5 mg/L, total suspended solids 157.25 mg/L, total dissolved solids 410.25 mg/L, free carbon dioxide 20.25 mg/L, total hardness 129 mg/L, calcium hardness 86.25 mg/L and magnesium hardness was 42.75 mg/L. All the parameters lie in optimum range except free carbon dioxide. The study adds 13 new records of fish species from Baran dam and shows its high fish diversity. It is suggested to take steps to control the siltation in Baran Dam to provide a better habitat for the fish.

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### PREVALENCE OF FISH DISEASES IN CULTURABLE FISH SPECIES OF PUNJAB

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This study was carried out to investigate the occurrence of different fish diseases in selected culturable fish species in various districts of Punjab Province. Total thirty nine (39) diseased fish samples belonging to seven (7) fish species viz., *Labeo rohita*, *Ctenopharyngodon idella*, *Cirrhinus mrigala*, *Catla catla*, *Hypophthalmichthys molitrix*, *Cyprinus carpio* and *Oreochromis niloticus* were randomly collected from eight (8) districts of Punjab viz., Lahore, Multan, Kasur, Sheikhpura, Sargodha, Faisal abad, Khanewal and Mandi Bahauddin. Total four (04) diseases were observed i.e., dropsy, hypoxia, learnesis and fin rot, during the investigations and the percentage of occurrence of these diseases was calculated as 52.4, 28.6, 14 and 4.8 percent respectively. Frequency of diseases in districts Lahore and Sheikhpura was calculated as 59% and 10.2% respectively. However, it remained at par for Faisalabad and Khanewal i.e. 7.7%. Similarly, in other two districts, Sargodha and Mandi bahauddin, it was observed as 5.1%. In districts Multan and Kasur, the trend was also similar as 2.6%. The frequency of all four fish diseases was found in order of dropsy>hypoxia> learnesis > fin rot while the prevalence these diseases in fish species under study was in order of *L. rohita*>*C. idella*, *H. molitrix*, *C. carpio* > *C. mrigala*, *C. catla*, *O. niloticus*.

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### EFFECT OF FERTILIZERS AND SUPPLEMENTARY FEEDING ON NITROGEN INCORPORATION AND BODY COMPOSITION OF FISH

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The aim of the present study was to to investigate effect of different fertilizers and supplementary feed on nitrogen incorporation and body composition of fish. Trials were conducted in earthen ponds having area of 0.02 ha each. There were 5 treatments and a control. Ponds were stocked with *Labeo rohita*, *Catla catla* and *Cyprinus carpio* in the ratio of 20:15:15, respectively. All the ponds received the same quantity of N but the sources were different. The amount of organic manure, fertilizer and supplementary feed was calculated on N-equivalence of 0.2g N/100g body weight of fish on weekly basis while supplementary feed containing 30 % C.P was administered daily. Control group received 100% nitrogen from organic manure (cow manure), treatment 1, from

inorganic fertilizers (nitrophos), 2, 50% nitrogen from cow manure and 50% from nitrophos, treatment 3, 50% from cow manure and 50% from supplementary feed, 4, 50% nitrogen from nitrophos and 50% from supplementary feed while treatment 6 received 25% nitrogen from cow manure, 25% from nitrophos and 50% from supplementary feed. Growth was assessed on monthly basis while proximate analysis was done only at the end of experiment. The overall range of nitrogen conversion ratio (NCR) varied from 1: 1.32 to 1: 9.65 in all the treatments. The maximum values were recorded in T<sub>6</sub> (9.65) followed by 9.30(T<sub>4</sub>). Statistical analysis for NCR revealed a highly significant difference for the months and non-significant for the treatments. Nitrogen incorporation efficiency (NIE) showed the seasonal variation from 0.104 to 0.765 in all the treatments. The best NIE was observed in T<sub>6</sub> (cow manure, nitrophos and supplementary feed) and T<sub>3</sub> (cowdung and supplementary feed) during January and August. Among three fish species, *Labeo rohita* showed the highest growth with the highest crude protein contents of 18.90% under the treatment T<sub>6</sub>, while *Cyprinus carpio* had the minimum crude protein content (15.62%) in T<sub>3</sub>. *Catla catla* showed the maximum total fat contents (2.02%) and *Cyprinus carpio* (2.00%) in T<sub>6</sub>. The lowest fat contents were observed in *Labeo rohita* in T<sub>1</sub> (1.05%). Overall Nitrogen Incorporation and protein retentions were better in T<sub>6</sub> when compared with other treatments.

#### ICHTHYOFAUNAL DIVERSITY AND WATER QUALITY ASSESMENT OF UPPER RIVER SWAT, KHYBER PAKHTUNKHWA, PAKISTAN

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Fish collection during this study was on track right from February 2013 to October of the same calendar year. During this study a total of 1156 fish specimens were captured with the assistance of various types of fishing nets, of various mesh sizes, like cast net and gill net. Moreover fishing tackles like hooks and rods were radically used. In this research survey total 19 species were reported which belongs to 3 families. Family Cyprinidae was the most booming family and represented by 11 species followed by family Nannochloridae consisted of 6 species whereas family Salmonidae was represented by only two species. Names of the species identified are *Schistura prashari*, *Schistura leptocheilus*, *Schistura alepidota*, *Schistura curtistigma*, *Crossocheilus diplocheilus*, *Carassius auratus*, *Triplophysa microps*, *Gambusia gotyla*, *Puntius conchoniensis*, *Glyptothorax maculatus*, *Oriens plagiostomus*, *Schizothorax plagiostomus*, *Racoma labiata*, *Barilius naseeri*, *Barilius pakistanicus*, *Barilius vagra*, *Labeo calbasu*, *Onchorynkiss mykiss* and *salmo trutta fario*. Water quality parameters were also investigated and the mean values of these parameters are; Temperature 14.63°C, pH 6.84, TDS 126.75 mg/L, TSS 49.13 mg/L, Alkalinity 114.38 mg/L, Salinity 0.04 mg/L,



Conductivity 195  $\mu\text{S}/\text{cm}$ , total hardness 103 mg/L, Ca Hardness 66.63 mg/L, Mg hardness 36.13 mg/L and Free  $\text{CO}_2$  7.25 mg/L. The study adds new records of fish species like *Carassius auratus*, *Gara gotayla*, *Puntius chonchonius*, *Acanthocobitis botia* and *Schistura alepedota* from upper Swat.

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**COMMUNITY STRUCTURE, ABUNDANCE AND BIOMASS OF FISHES IN  
VEGETATED AND NON VEGETATED INTERTIDAL ZONES OF MIANI HOR,  
SONMIANI BAY, BALOCHISTAN**

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Mangroves form a habitat for a wide variety of species, some occurring in high densities. Species compositions of fish assemblages have a great abundance and diversity in the mangrove. Estuarine habitats have been recognized as important drivers of nearshore fish productivity. Worldwide, about 30% of all commercial fish species are mangrove-dependent. Differences in fish composition among mangrove intertidal channel and adjacent non vegetated intertidal zones were subjected to studies, conducted over a year of time period in naturally occurring mangroves of Miani Hor (Sonmiani Bay), Balochistan. Studies on the fish fauna and community structure of vegetated and adjacent non vegetated sandy intertidal flats stressed that the majority of species use these systems as nurseries and/or foraging areas. Examples of species that occur in most mangrove areas of this region include the sea bream *Acanthopagrus berda*, the purse mouth *Gerres filamentosus*, the mangrove jack *Lutjanus argentimaculatus*, the flathead *Platycephalus indicus*, the flounder *Pseudorhombus arsius*, the whiting *Sillago sihama* and the thornfish *Terapon jarbua*. Fishes consisting of 22 species expressed their assemblage by 14 families that contributed 58.22% of the total sample by numbers collected through beach seine from sandy substratum. There were 14 species of fishes constituted 71.46% of faunal assemblage at sandy cum muddy shallow intertidal habitat. Almost 70.74% fishes were found in abundance as compared with shrimps (19.80%) and crabs (9.45%) in the Northeast monsoon. There were a few species of fishes with numerous individuals dominated (90.78%) the southwest monsoon faunal composition in Bhaira (mangrove dominated habitat/muddy substratum). These mangroves and adjacent shallow zones are characterized by stable salinities and their ichthyofauna is dominated by juveniles.

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**ICHTHYOFAUNISTIC DIVERSITY AND ASSESSMENT OF WATER  
QUALITY OF RIVER KURRAM, DISTRICT BANNU, KHYBER  
PAKHTUNHWA, PAKISTAN**

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The present study of river Kurram, District Bannu, K.P., Pakistan was conducted for six months from January 2013 to June 2013 to find out its ichthyofaunistic diversity and water quality. Different fish gears like cast nets, drag nets, meshed cloths and the technique of temporary draw down were used for fish collection. During the study 24 species of fishes were collected and identified from River Kurram. Family Cyprinidae was the most dominant one represented by 15 species viz., *Aspidoparia morar*, *Barilius vagra*, *Barilius pakistanicus*, *Barilius naseeri*, *Barilius modestus*, *Cirrhinus mrigala*, *Cirrhinus reba*, *Crossocheilus diplocheilus*, *Chela laubuca*, *Gara gotyla*, *Labeo dyocheilus*, *Puntius ticto*, *Puntius sophore*, *Puntius conchoni* and *Systomas sarana*. Family Nemacheilidae was represented by 3 species viz., *Nemacheilus corica*, *Schistura prashari* and *Triplophysa microps*. Family Sisoridae was signified by 3 species namely *Glyptothorax punjabensis*, *Glyptothorax cavia* and *Glyptothorax sufii*; Family Siluridae, Mastacembelidae and Cichilidae were represented by only one species each viz *Ompok pabda*, *Mastacembelus armatus* and *Oreochromis aureus* respectively. The mean values of some physico-chemical parameters of water of River Kurram were; Temperature 25.33°C, speed of water 1.51 m/sec, pH 6.83, TDS 279.39mg/L, TSS 175.05mg/L, Conductivity 464.83 µs/cm, Salinity 0.23 ppt, Free CO<sub>2</sub> 18.55mg/L, Total hardness 157.72 mg/L, Ca hardness 109.39 mg/L and Mg hardness 41.66 mg/L. The study adds four new records to the previous list of fishes reported from the river Kurram viz. *Chela laubuca*, *Barilius naseeri*, *Glyptothorax sufii* and *Oreochromis aureus*. All the studied water parameters fall in standard range except Free CO<sub>2</sub> and Mg Hardness that are above the normal range suggested by United States Public Health standard for surface water in 1976.

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**STUDIES ON THE EFFECTS OF SEEPAGE PROBLEM ON THE PRIMARY  
PRODUCTIVITY AND FISH GROWTH IN EARTHEN PONDS**

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Three fish species *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* were grown in

earthen ponds for 8 weeks under two treatments (with two replicates of each) designated as T<sub>1</sub> & T<sub>2</sub>. T<sub>1</sub> had high seepage rate as compared to T<sub>2</sub>. During the course of study all experimental ponds were fertilized. Rate of seepage of the ponds under both treatments was determined by using meter rods fixed in the ponds on daily basis. The average rate of seepage in T<sub>1</sub> remained in the range of 7.1± 0.85 to 12.3 ± 0.49 cm while in T<sub>2</sub> it remained in a range of 23.3± 3.96 to 28.5 ± 5.09 cm per day. Quantitative estimation of plankton life of the ponds under two treatments in terms of numbers /ml was also carried out with the help of Sedgwick Rafter Counting Chamber. Range of phytoplankton under T<sub>1</sub> remained of 1129.6 ± 2199.81 to 5073.0 ± 1858.26/ml while under T<sub>2</sub> it remained in a range of 249.9 ± 210.41 to 1332.8 ± 1092.31 /ml. On the other hand, the range of zooplankton count in T<sub>1</sub> remained in range of 2.8±0.69/ml to 3.5±0.66/ml while in T<sub>2</sub> it was 0.2±0.1 to 0.1±0.01/ml. Growth of *Catla catla* in terms of weight was significantly (p< 0.05) higher in low seepage ponds than in higher seepage ponds. Growth of *Labeo rohita* also showed similar trend of significant (p<0.05) difference between two treatments. However, *Cirrhinus mrigala* showed statistically non-significant difference between two treatments. It is concluded that seepage exerts significant impact on the planktonic productivity of ponds that ultimately shows negative impact on the fish growth specially that of *Catla catla* and *Labeo rohita*, while the impact of seepage remained non significant upon the growth of *Cirrhinus mrigala*.

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**COMPARATIVE EFFECT OF THREE STRAINS OF DIETARY PROBIOTICS  
GEOTRICHUM CANDIDUM ON GROWTH PERFORMANCE AND IMMUNITY  
OF CTENOPHARYNGODON IDELLA AND TOR PUTITORA**

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The present study was conducted to evaluate the comparative effect of three strains of dietary probiotics *Geotrichum candidum* viz., UCMA-91, 103 and 322 on growth performance and immunity of juvenile *Ctenopharyngodon idella* and *Tor putitora*. Experiment was conducted in replicate in glass aquaria for 90 days. Three 35% protein diets supplemented with three tested strains at a concentration of 1009 CFU kg<sup>-1</sup> diet and fourth basal diet without supplementation were fed to *C. idella*. Similarly, 40% protein basal diet and three experimental diets enriched with same strains and concentration of probiotics were offered to *T. putitora*. Significantly higher % weight gain, improved SGR and FCE was observed in groups of *C. idella* fed on UCMA-322 supplemented diet as compared to *T. putitora* which showed better performance with

UCMA-103 enriched diet. Results of the challenge test with *E. coli* indicated clear symptoms of enteritis with low lysozyme activity in both species raised on basal diet while significantly higher lysozymal activity was observed in group of *C. idella* and *T. putitora* fed on UCMA-322 and UCMA-103 supplemented diet respectively. The results of study reveal the strain base effectiveness of probiotics in different fish species.

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### EFFECT OF DIFFERENT PIGMENTS ON THE COLOUR DEVELOPMENT OF GOLDFISH AND RED SWORDTAIL

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The experiment was carried out to determine whether carotenoids of orange peels could induce pigmentation to make a red sword-tail and goldfish more colorful. Completely randomized design was used in this experimental trail. The experiment was carried out in 10 plastic troughs of 30 lit capacities for 30 days. These troughs were divided into 2 groups and each group contains 5 troughs. Group one (Goldfish) has 5 treatments including control viz. G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, G<sub>5</sub> troughs. Similarly group two (Swordtail) had 5 treatments viz S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub>, and S<sub>5</sub>. G<sub>1</sub> in group one and S<sub>1</sub> in group two served as control in respective trials. In each group five fish were randomly allowed in each replicate of each treatment group. Dried orange peels in powdered form were used as pigment enhancer. TLC method was used for pigment extraction from orange peel. The experimental diet composed of basic ingredients like Fish meal, Cotto<sup>^</sup> seed meal, maize gluten and rice polish in the ratio of 12:8.925:6: 2.499; 12:6:6:1,249; 10:6:4.5:1.249; 12:2.975:3:1.249 and 12:1.48:3:0.624 in five experimental trail. They were mixed with different quantities of orange peels in the ratio of 5.25: 7.875: 10.5: 13.1 at 20, 30, 40 and 50% protein levels in G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, G<sub>5</sub> and S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub>, and S<sub>5</sub> respectively. R<sub>f</sub> values were calculated by the measurement of the distances from the starting point to the solvent front and from the starting point to the center of each spot. Result showed that the R<sub>f</sub> value of different TLC plates; In plate 1, spot 1 travel maximum distance (0.88) (Red-Orange) and have highest R<sub>f</sub> value, similarly in plate 2 (0.77) (Yellow – brown), spot 2 and in plate 3, spot 2 travel maximum (0.90)( Yellow - orange ) distance and have highest R<sub>f</sub> value under UV lamp. In this experiment, Plate I showed Lycopene, Xanthophyll, Plate II showed Leutein, Violaxathin, Pheophytin and plate III showed β-carotene, carotene, Xanthophyll. The results indicated that the carotenoid content in G<sub>5</sub> and G<sub>4</sub> was higher than control group at 380 nm. Carotenoids are the primary source of pigmentation in ornamental tropical fish, responsible for various colors like yellow, red and other related colors and the maximum carotenoid contents was present in the fish fed with 50% orange peel contents. Statistical analysis showed the significant differences among average values of total carotenoid content.

Results showed that orange peels were found to be effective color enhancer at a cheaper price.

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**POPULATION ESTIMATION OF AN AQUARIUM FISH *CHANDA RANGA* IN AN EDIBLE FISH POND AT THATTA, SINDH**

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*Chanda ranga* is internationally demand aquarium fish. It is thickly populated in Sind and Punjab Province of Pakistan. Our rival country India is exporting it to main international market of Far East. No effort has yet been made to export it from Pakistan. It is mainly due to the reason that the no effort has yet been made to explore the biodiversity of this fish. Therefore, in this study, by the probability method it is tried to find the total population of this freshwater glass fish in a 4 acre pond of edible fish. They use to grow in such ponds in Pakistan. 5 samples of 100 fishes were collected, where each sample contains 100 fishes. These samples were marked with red, blue, orange and green color. Where, one sample of 100 fishes was not marked. The hand net of 40 feet long and 12 feet height was used. The mesh size of net was 0.25 centimeter. The average depth of pond was 3 feet, during the experimental month. Every sample was taken after the break of 7 days. The collected fishes were injected florescent color with BD Needle, soon after the collection. The marked fish was kept in aquarium of having size w=12", Length=60" and height=18". The injected fishes were treated with chloramphenicol capsule. Fishes was again introduced into to pond under experiment. This process was done after 5 days aquarium rearing. During these 7 sampling following results were obtained. The 1 00 pieces of 1st sample was marked with red color at face side of the dorsal fin. In the 2<sup>nd</sup> sample of 100 fishes, 88 were unmarked and 12 are already marked with red color. The 88 unmarked fishes are marked with blue color and rest of 12 fishes are also 2<sup>nd</sup> time marked with blue color at tail end part of dorsal fin. In the 3<sup>rd</sup> Sampling 74 was found un-marked, 16 was blue marked, 3 are red marked and 7 are red and blue marked fishes. The 74 un marked fishes are now market with orange color at the first of dorsal fine. 16 blue marked color fishes of 3<sup>rd</sup> sample are now also marked with orange color at the tail part of dorsal fin. 3 red marked collected fishes are now also marked with orange color at the tail part of dorsal fin. 7 red & blue marked collected fishes are now marked with orange color at the mouth side of anal fine. In the 4th sample of 100 fishes, the 38 unmarked fishes were collected and they were marked with green color at the first par of dorsal fine. 12 fishes of red color are collect, 7 fishes of blue color was collected. 7 fishes of orange color were collected. 18 fishes of red, blue & orange colored was collected. Similarly, up to 7th sample same procedure was followed. Hence, by the application of probably and central tendency theory on this data, the total population of glass fish (*Chanda ranga*) is estimated. A total of 13,570 fishes were present in this pond.

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### **AGGLOMERATIVE HIERARCHICAL CLUSTERING BASED ON MORPHOMETRIC PARAMETERS OF THE POPULATIONS OF *LABEO ROHITA***

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*Labeo rohita* populations from five geographical locations from the hatchery and Riverine system of Punjab-Pakistan were studied for the clustering on the basis of similarities and differences based on morphometric parameters within the species. Agglomerative Hierarchical Clustering (AHC) was done by using Pearson Correlation Coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as Agglomeration method by XLSTAT 2012 version 1.02. A dendrogram with the data on the morphometrics of the representative samples of each site divided the populations of *Labeo rohita* in to five major clusters or classes. The variance decomposition for the optimal classification values remained as, 19.24% for within class variation while 80.76% for the between class differences. The representative central objects of the each class, the distances between the class centroids and also distance between the central objects of the classes were generated by the analysis. A measurable distinction between the classes of the populations of the *Labeo rohita* was indicated in this study which determined the impacts of changing environment and other possible factors influencing the variation level among the populations of the same species.

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### **REPLACEMENT OF FISHMEAL WITH SELECTED PLANT BY- PRODUCTS IN ARTIFICIAL FEED OF ROHU (*LABEO ROHITA*)**

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A 90-day feeding trial was conducted to determine the effect of replacing fishmeal with plant by products (soybean meal, cotton seed meal, guar meal, rice polish, canola meal) on growth performance and blood chemistry of *Labeo rohita* with and without fish meal inclusion in both pelleted and powder form which were hereafter designated as control, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> with four replicates in each. Fish were housed in fiberglass tanks with stocking density of 126.05mg/liter. The studies were continued for 90 days. Control diet was from Oryza Organics Lahore and had 30% protein. Diet 1 was based on fish meal powder while diet 2 was without fish meal powder. Diet 3 and 4 were similar to diet

1 and 2 respectively but were pelleted before feeding to fish. Fish were fed @ 5% wet biomass of fish. Initial average weight of control group, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> was 224.700±0.10<sup>c</sup>, 337.750±0.050<sup>a</sup>, 314.17±0.01<sup>d</sup>, 361.1±0.001<sup>e</sup>, 212.705±0.005, respectively. Dissolved oxygen and temperature were checked daily there was negative but significant correlation between them in T<sub>1</sub> and T<sub>3</sub> which was fed by fishmeal powder and pellets. Nitrate and phosphates were determined on every third day while survival and body weight increments were measured on every fortnight. There was positive correlation between phosphates and nitrates with treatment T<sub>1</sub> and T<sub>3</sub> but phytoplankton production correlated negatively with these dietary treatments. Survival and growth rate remained same means that locally formulated feed was comparable with that of the commercial feed. In organoleptic test color, flavor, juiciness, oiliness and overall acceptability were significantly higher in feed 1 where fish was included in diet and tenderness was high in fishmeal pellets (T<sub>4</sub>). Red blood cells and white blood cells were significantly higher in Group T<sub>1</sub> than all its counterparts. Feed was analyzed for Aflatoxins but were not detected.

#### FEEDING HABITS OF CYPRINIDAE AND SALMONIDAE IN RIVER SWAT, KPK, NORTHERN PAKISTAN

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Studies on food and feeding habits of fishes offer a great deal of information regarding seasonal changes in fish fauna as well as their life cycles. Therefore the current study was conducted to know the feeding preferences of *S. plagiostomus* and *S. esocinus*, belonging to family Cyprinidae, and *S. trutta fario* of family Salmonidae from River Swat, Khyber Pakhtunkhwa. The river Swat was divided into five station namely Kalam, Behrain, Madyan, Fatehpur and Khwaza Khela. Fish were sampled from the mentioned stations month wise from May to December 2012. Populations of different orders of insects were compared month and station wise. *S. trutta fario* was found with broad range of feeding diversity of aquatic and terrestrial insects available in its gut content. Order Diptera was found to be the most abundant (29%), followed by Tricoptera (19%), Plecoptera (18%), Hemiptera (17%), Ephemeroptera (14%), Hymenoptera (3%) and Coleoptera (1%). The insects available in gut of *S. plagiostomus* were order Diptera (91%) and Detritus (9%). The food of *S. esocinus* was composed of order Diptera (75%) order Tricoptera (12%), Detritus (10%), and order Coleopteran (4%). High values for Shannon-Weiner index and Simpson's diversity index were observed at Kalam whereas Pielou's evenness index was observed at Madyan. The most consumed prey was the Diptera. The least consumed prey was the Coleoptera among the targeted species. This study highlights the importance of Dipteran as a food source for the cold water fishes.

Dipteran conservation is recommended on the basis of current study for conservation of cold water fishes.

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**AGE DETERMINATION OF MINOR CARP, *CIRRHINUS REBA* (HAMILTON)  
FROM MANCHAR LAKE, SINDH, PAKISTAN**

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For age determination study of *cirrhinus reba* (Hamilton) from Manchar, length-frequency analysis and scale method was used. It was determined from 256 specimens (171 male and 85 female) collected during March to June 2013 ranging in length between 95 to 330 mm in total length. The entire data were pooled, male, female and their combined length-frequencies as percentage. It was inferred from the length-frequency analysis that the model length of group 10.0-20.0 cm represents as 0+ age group and the subsequent model lengths 20.0- 30.0 cm designated as 1 + age group respectively. The peaks of frequency histogram, beyond two years were not found to be distinct. The relationship between total length versus scale length was found to be linear  $\text{Log } L = 0.78 + 0.95 \text{ Log } S$ . The age estimate from scales resulted in to two groups 0+ and 1 + at model length 17.5 and 28.0 cm. The age determination from length-frequency and scale method compared very well.

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**CATFISH BIODIVERSITY AND PRODUCTION OF LOWER INDUS RIVER,  
DISTRICT THATTA, SINDH, PAKISTAN**

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Studies on biodiversity of catfish were conducted during August 2010 to April 2011. The fish data were collected from six different stations of lower Indus River, district Thatta. These included Thatta, Chuhar Jamali, Sajawal, Garho, Jati and Mirpur Sakro. A total of 12 catfish species namely *Bagarius bagarius*, *Rita rita*, *Heteropneustes fossilis*, *Clupisoma garua*, *Eutropiichthys vacha*, *Clarius batrachus*, *Sperata seenghala*, *Ompok bimaculatus*, *Ompok pabda*, *Mystus cavasius*, *Mystus vittatus* and *Wallago attu* were recorded. The catfish production from six sampling stations was also documented during the study period. The annual catfish production from Thatta Market contributed 319 metric tons (m.t). The highest fish production at Thatta was 37.65 m.t (August), while lowest 16.84 (December). Mirpur Sakro contributed 94 m.t fish. The maximum production was 14.45 m.t (September) and lowest 4.36 m.t (January). The sampling station Chuhar Jamali produced 73 m.t. The maximum fish production was 8.56 m.t (September), while minimum 4.49 m.t (January). The fish production of Sujawal was



48.96 m.t. The highest fish production was 5.16 m.t (August), while lowest 2.93 m.t (January). Jati contributed 45.67 m.t fishes. The highest fish production was 7.5 m.t (September) and lowest 1.69 m.t (January). The minimum fish production was recorded from Garho. It produced 32.18 m.t., maximum being 5.74 m.t (September), while minimum 1.05 m.t (January). Catfish *W. attu* was on the top in terms of production whereas *Rita rita*, *Sperata seenghala* and *Eutrophichthys vacha* were good contributors. Documentation of catfish biodiversity and production in the proposed study was utmost important for the commercial fishery.

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### **SOME OBSERVATIONS ON THE SEASONAL DISTRIBUTION AND ABUNDANCE OF BILLFISHES ALONG THE PAKISTAN COAST**

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Billfish forms an important part of the bycatch of tuna gillnetting in Pakistan. Six species of billfish belonging to six genera and two families of billfishes are reported. Of these, one species *i.e. Xiphias gladius* belongs to family Xiphiidae whereas all other species belonged to family Istiophoridae. Annual landing of billfishes is estimated to be about 3,700 m. tons. Although data pertaining to species composition is not recorded officially but studies indicate that Indo-Pacific sailfish is the most dominating species of billfish found in Pakistan followed by black marlin and striped marlin. Swordfish is the rarest of all the billfishes which is seldom caught by pelagic gillnetters. Billfishes are not consumed in Pakistan, therefore, major part of the catch is smuggled to Iran whereas small quantities, in salted-dried form, is exported to its traditionally market in Sri Lanka. The paper presents results of the recent data collection regarding fishing ground, species composition, population parameters and other aspects of billfish fishery. It was observed that the seasonal abundance correlates significantly with the prevailing oceanographic conditions in the northern Arabian Sea.

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### **EFFECT OF AQUACULTURE FEEDING PRACTICES ON COMMERCIAL FISH FARMING IN RAWALPINDI DISTRICT**

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A survey of private fish farms was carried out to study the effect of aquaculture feeding practices in Rawalpindi district. Fifteen private fish farms were randomly selected, out of them five farms used balanced supplementary feed (Rice polish, Fish meal and Soybean meal), five farms used standard feed (Rice polish) and five farms used conventional feed (Fodder) to feed the fish under polyculture system. The average

stocking rate of fish was 1200 fingerlings/acre with average weight of 4 grams. The fingerlings were fed with constant feeding rate of 3% of their body weight. Highest production obtained with balanced supplemented feed followed by standard feed and conventional feed for *Labeo rohita* (373.56, 344.26 and 286.40 kg/acre/year respectively). *Cirrhinus mrigala* showed higher production with balanced supplemented followed by standard feed and conventional fish feed (304.22, 282.71 and 207.22 kg/acre/year), *Ctenopharyngodon idella* gave highest production with balanced supplemented feed (361.24 kg/acre/year) and less production with both standard and conventional feed (212.15 and 205.31kg/acre/ year) while the difference of *Hypophthalmichthys molitrix* production was non significant for all of three types of diets. The outcome shows that the use of balanced supplemented feed (fish meal, rice polish and soybean) will help in better production in major carp under polyculture system.

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#### **EFFECT OF PLANT BASED PROTEIN LEVELS ON GROWTH PERFORMANCE OF A CHINESE CARP**

NARGIS TASLEEM AND MUHAMMAD ZUBAIR ANJUM

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A 60 days experiment was carried out in six glass aquaria to study the effect of replacement of fish meal by plant based protein (Soybean meal and Canola meal) *i.e.* 25%, 50% and 75% in fish diet on the growth performance of silver carp. The fingerlings were fed at 5% of the wet body weight once daily and each diet contained 30.9% crude protein. The comparison of three plant based protein replacements of fish meal in fish feed resulted in maximum body weight gain in grams ( $9.950 \pm 0.39$ ) with 25% replacement followed by 75% and 50% *i.e.*  $9.611 \pm 0.37$  and  $8.674 \pm 0.25$  respectively. Analysis of variance showed a significant difference between all the three different diets. The survival rate was 100% and water quality conditions were optimum for the growth of fish. Overall results revealed that some of the oilseed proteins have the potential to be used as partial substitute to the fish meal for the carp culture which helps in reducing the financial burden on fish farmers.

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#### **FISHERIES POTENTIAL AND LIMNOLOGY OF LAKE GAHOT, MATIARY, SINDH, PAKISTAN**

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Lake Ghahot is located 25°56'04.70"N and 68°25'55.44"E on the global map. Locally it is situated near Simroon bank of Indus river near to village Allah Rakhyo

Mallah in Saeedabad, district Matiary, Sindh. It covers the area of about 200 acres and depth is about 25-32 ft. The lake receives water directly from Ghahot canal and escape of Indus river during flooded season to the southern regions. Water quality and biological samples were collected and studied on seasonal basis. Temperature of water was found between 16 to 34°C, visibility in water was observed 0.7 to 1.3 m, pH ranges were observed 8.4 to 8.9; alkalinity and hardness (CaCO<sub>3</sub>) were noted 198 to 212 mg/l and 217 to 320 mg/l respectively. Salinity, conductivity and TDS were noted 1.09 to 2.8 ppt, 2141 to 5250 µS/cm, and 1204 to 2573 mg/l respectively; dissolved oxygen ranges were recorded from 7 to 12.5 mg/l. 32 species of phytoplankton and algae belong to phylum Cyanophyta, Chlorophyta and Bacillariophyta were recorded. The filamentous attached floating genera such as *Cladophora*, *Mougeotia*, *Oedogonium*, *spirogyra* and *Hydrodictyon* were commonly found at shallow areas. 11 species of zooplankton were observed, among them the *Diaphanosoma*, *Simocephalus*, *Mesocyclops*, *Cyclops*, and *Neodiaptomus* were found dominant during spring and summer season. 32 species of fish consisting the group of carps, catfishes, spiny eels, snake heads, tilapia were reported. The total catch from all landing centers was 45.5 metric tons/annum. The fish production and natural productivity of lake Gahot is rich in relation to its limnological parameters.

#### LENGTH-WEIGHT RELATIONSHIP OF FISH SPECIES IN RELATION TO LIMNOLOGY OF RIVER INDUS AT DISTRICT D. I. KHAN, PAKISTAN

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The present study deals with physico-chemical properties of water and length-weight relationship of three fish species (*Cirrhinus mrigala*, *Labeo rohita* and *Rita rita*) from river Indus at district D. I. Khan, Pakistan. Various physico-chemical properties such as (Temperature, pH, EC, Salinity, TSS, TDS, DO, Chloride, Carbonates and Bicarbonates) were recorded on monthly basis from river Indus which ranged between 32.13±0.15 to 23.10±0.10 (°C), 8.11±0.14 to 7.27±0.07, 329±2.08 to 192±3.61 (µS/cm), 0.23±0.06 to 0.03±0.06 (‰), 533±3.21 to 37±3.51 (mg/l), 907±3.21 to 164±2.65 (mg/l), 8.88±0.02 to 6.77±0.13 (mg/l), 33±1.53 to 6±0.57 (mg/l), 56±1.53 to 11±1.53 (mg/l) and 110±2.08 to 19±0.58 (mg/l) respectively which were within permissible limits for aquatic diversity except TSS. The length-weight relationship of were calculated for *Cirrhinus mrigala*, *Labeo rohita* and *Rita rita* at upstream, sewage site and downstream. The relationship was analyzed using the formula  $W = aL^b$  which was further transformed into  $\log W = a + b \log L$ . A total of 18 samples of *C. mrigala*, *L. rohita* and *R. rita* were

collected from one site, thus from three sites total number of samples were 54 for each species. The equations obtained for *C. mrigala*, *L. rohita* and *R. rita* were at upstream:  $\text{LogW} = -3.9145 + 2.708 \log L$ ,  $\text{LogW} = -3.6223 + 2.565 \log L$  and  $\text{LogW} = -4.1114 + 2.681 \log L$ , at sewage site:  $\text{LogW} = -3.9776 + 2.738 \log L$ ,  $\text{LogW} = -3.4462 + 2.490 \log L$  and  $\text{LogW} = -3.8588 + 2.575 \log L$ , at downstream:  $\text{LogW} = -4.0594 + 2.768 \log L$ ,  $\text{LogW} = -3.5304 + 2.527 \log L$  and  $\text{LogW} = -3.9169 + 2.597 \log L$  respectively which shows negative allometric growth. The sewage water has no effect on the growth as there was no difference in equations between three sites and all the water quality parameters were within permissible limits except TSS.

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#### **EVALUATION OF GROUND WATER QUALITY OF TEHSIL BARNALA, DISTRICT BHIMBER, AJK, PAKISTAN**

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Present study was designed to obtain an estimation about the ground water quality of Tehsil Barnala. A total of 7 water samples were collected from Tehsil Bhimber to analyze for various physicochemical and biological parameters *i.e.* namely temperature, pH, Turbidity, Color, Odor, taste, Electric Conductivity (EC), Total Dissolved Solids (TDS), Total Hardness (Calcium + Magnesium), Chloride, Arsenic, Nitrite and *Escherichia coli*. Results revealed that ground water of District Bhimber is grossly contaminated with pathogenic micro organisms like *E.coli*. Besides it, values of some physicochemical water quality determining parameters were also beyond the limits suggested by World Health Organization (WHO) *i.e.* Chloride ion concentration was below the prescribed limits. It has been proven that consumption of un safe drinking water is one of the major cause of prevalence of water born diseases like diarrhea, typhoid fever and malaria etc in the study area. It is stated that ground water of Tehsil Barnala is not suitable for drinking without treatment.

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#### **AN ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN DRINKING WATERS OF SULEMAN RANGE MOUNTAINS**

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The levels of heavy metal contamination in various drinking water resources of Suleman range mountains and the western bank of the Indus River in District Dera Ghazi Khan were ascertained for heavy metals toxicity. The incidence of heavy metal

contamination in ground water used for human consumption has taken the dimension of epidemiological problem in mountainous Suleman ranges. It has also been established that these heavy metals are extremely toxic both acute and chronic. The study examines the potential health risk caused by consumption of heavy metals to local inhabitants living in the district. A some of 75 drinking water samples were analyzed for heavy metals including Arsenic, Chromium hexavalent, Copper, Iron, Fluoride and Zinc concentrations. Concentration of above mentioned metals scrutinized that some samples having more concentration than guideline values, hence these sources are not suitable for drinking purposes. Therefore it is recommended that drinking water should be regularly monitored for heavy metals in order to prevent excessive buildup of these heavy metals in the human food chain.

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### 3. MARINE BIOLOGY

#### A CHECKLIST OF AQUARIUM CLEANING CRABS OF KARACHI COAST AND THEIR CAPTIVE TOLERANCE LEVEL

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In Pakistan seawater aquarium keeping practice is increased reasonably during the last decade. Increasing use of seawater aquariums is opening many challenges for marine scientists. Of these biological cleaning, is one the most important scenario. In this study, to overcome this problem the authors are introducing 10 species of small crabs and 3 species of hermit crabs to work as cleaning crew in the marine aquariums. Our 10 year observation reveals that the aquarium's water polluted as a results of increase in ammonia. This ammonia is due to left over foods and death of aquatic life. These cleaning crews are observed feeding on dead animals and left over food. Hence, these cleaning crews do not these two parameter moves toward the disturbance of aquarium's eco system. It is found that these cleaning crews leave a live for 2 months, without any food. The salinity tolerance range is 10 ppt to 40 ppt of tinny crabs. The salinity tolerance ranges of hermit crabs are 5ppt to 40ppt. Where, temperature tolerance level is 10 to 40 degree centigrade. They are also calculated cheaper, as compare to other available tools to control water chemistry of marine aquarium.

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#### DETERMINATION OF BIOACCUMULATION AND BIOCONCENTRATION FACTOR IN SOME BRACHYURAN CRABS FOUND ON INTERTIDAL MUDFLATS OF PAKISTAN

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Heavy metals can be believed as potential noxious wastes of the coastal environment and their monitoring can be accomplished by the substrate and their fauna analysis. The current study was conducted to investigate the bioaccumulation and bioconcentration factor (BCF) of eight heavy metals in seven brachyuran crab species namely, *Ilyoplax frater*, *Opusia indica*, *Uca iranica*, *U. sindensis*, *Scopimera crabriacauda*, *Macrophthalmus bosci* and *Eurycarcinus orientalis*. The sediment and crab samples were collected at low tide by quadrat method from Sandspit, Korangi Creek, Port Qasim, Rato Kot, Sonari and Sonmiani during June 2011 to Jan 2012. The results indicated that crab species accumulate a considerable amount of metals in their body which was significantly different among the sites and species. The mean Bio

Concentration factor (BCF) of metals in all studied crab species obtained for Ni, Fe, and Cr were less than 1.00, usually considered as no bioaccumulation. However, BCF greater than 1.00 were obtained for Co, Zn, Pb, Cu and Cd evidently indicating that these metals are accumulated and biomagnified. The order of Bio-concentration Factor for heavy metals found in crab species are as, Cu>Co>Cd>Zn>Pb>Fe>Cr>Ni in *O. indica*, Cu>Cd>Co>Zn>Pb>Ni>Fe>Cr in *I. frater*, and Cd>Pb>Cu>Ni>Zn>Co>Cr>Fe in *U. sindensis*.

**THE MORPHOLOGICAL DIFFERENCES AND ISOZYME VARIATIONS  
BETWEEN THE TWO SPECIES OF *ILYOPLAX* (DECAPODA, BRACHYURA,  
DOTILLIDAE) FOUND IN MANGROVE AREAS OF PAKISTAN**

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Crabs of genus *Ilyoplax* Stimpson, 1848 are ecologically important and numerically dominated members of mangrove ecosystem throughout the world. The present study based on morphological differences and genetic aspects of two species of *Ilyoplax* (*Ilyoplax frater* and *Ilyoplax stevensi*) found along subtropical coastline of Pakistan. The two species can be distinguished on the basis of some morphological characters (Carapace shape, its proportions, Chela shape and dentition, male pleopod). For genetic variations, vertical polyacrylamide gel electrophoretic technique was adopted. Isozyme screening was carried out for three enzymes *i.e.* Carbonate dehydratase (CD), Catalase (CAT) and Creatine Kinase (CK). The results reveal that Catalase showed species specific pattern for 2 loci out of 3, locus 1 is polymorphic with observed heterozygosity  $H_o = 0.2$  for *Ilyoplax stevensi* and  $H_o = 1$ , for *Ilyoplax frater* respectively. The species-specific genetic marker (banding pattern) was observed with Rf values of 0.771 and 0.751 for *Ilyoplax stevensi* and *Ilyoplax frater* respectively.

**INFLUENCE OF HETEROGENEOUS BIOTOPES ON DISTRIBUTION AND  
DENSITY OF CRAB BURROWS ALONG THE COAST OF PAKISTAN**

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Crabs are a significant component of benthic fauna and considered as an ecosystem engineer because of their dynamic role as a burrower in coastal mudflats. The current investigation is to evaluate the density and distribution of crab burrows by the transect quadrat method during low tide along the nine sites (Ketibunder, Dhabeji,

Bhambore, Phitti creek, Korangi creek, Sandspit, Sonari, Sonmiani and Bhaira) of Pakistan. The granulometric analyses were also carried out to determine the heterogeneity of biotopes. Our results showed that significant variations were observed in density and distribution of crab burrows according to the biotope heterogeneities ( $F_{2,91}=6.53$ ,  $P=0.000$ ) but not for tidal levels. Burrow diameter were significantly different for sites ( $F_{2,91}=23.11$ ,  $P=0.000$ ) as well as for tidal levels ( $F_{2,91}=20.38$ ,  $P=0.000$ ) revealed the fact that variations in crab populations and niche preferences occurs among different crab communities. Regression analysis showed that crab density was significantly correlated with burrow density implying the fact that burrow counting can be a valid or a suitable procedure to estimate crab densities along the coast of Pakistan. The Pearson correlation matrix was used to elucidate the relationship between sediment, crabs and their burrows. The relationship between the crab burrows with biotope heterogeneities further magnified by the application of principle component analysis.

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**MORPHOLOGICAL AND GENETIC COMPARATIVE DIAGNOSIS OF TWO SPECIES OF PORTUNID CRAB *PORTUNUS PELAGICUS* (LINNAEUS, 1758) AND *PORTUNUS SEGNIS* (FORSKAL, 1775) FROM THE COASTAL WATERS OF PAKISTAN**

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*Portunus pelagicus* blue swimmer crab is reportedly widespread throughout the Indo-West Pacific region and found as a complex of four morpho type. Based on morphological, morphometrical and genetic analysis revealed that two species *Portunus pelagicus* and *Portunus segnis* reside in the coastal waters of Pakistan. The *P. segnis* considered as new report during present study. The *P. pelagicus* and *P. segnis* were identified on few morphological differences. In the present study 23 external morphometric characters were taken and standardized by using ratios. Stepwise discriminate analyses (DA) were performed to determine groupings. The eight variables were tested, only one was significantly different in single character ratios. One-way ANOVA analyses of ratios among males of *P. pelagicus* and *P. segnis* revealed significant differences in Natatory leg dactylus length to Natatory leg dactylus width (NDL/NDW) and ( $df = 1$ ,  $F = 5.07$ ;  $P < 0.05$ ). In genetic analysis five enzyme systems viz. Carbonate Dehydratase, Catalase, Creatine Kinase, Octanol Dehydrogenase and Glucose -6- Phosphate Dehydrogenase were studied. The results of statistical comparisons were coincident that external morphometric characters and genetic analysis between the *P. pelagicus* and *P. segnis* were significantly different.

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**PRELIMINARY EVALUATION OF THE HEALTH RISK ASSESSMENT OF  
HEAVY METALS CONTAMINATION IN CRAB SPECIES FOUND ALONG  
THE KARACHI COAST, PAKISTAN**

NAYAB KANWAL AND NOOR US SAHER

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The present study was conducted to investigate the contamination level of some heavy metals (Cu, Cd, Zn, Cr, Fe, Pb, Ni) in four edible crab species by acid digestion through an atomic absorption spectrophotometer. The dietary intake and potential human health risk associated with the consumption of contaminated seafood/shellfish were estimated. Generally the presence of contaminants in seafood is a result of human activities such as incorporation of industrial and agricultural wastes in sea water. The present research determined the concentrations of Zn and Pb were comparatively higher in the studied commercial edible species of the family Portunidae. In *Scylla serrata* the highest concentrations of Zn ( $232.4 \pm 257.5$  mg/kg) and Ni ( $75.1 \pm 59.3$  mg/kg) were observed in the edible portion. As a food item the concentration level of all the analyzed metals were under permissible range except Pb as the concentration was higher than the limit proposed by FAO. The estimated daily intake values of these metals through human consumption were calculated (mg/kg body wt/day or per week) and compare with those of provisional tolerable weekly intake (PTWI) per Kg body weight as set by the FAO/WHO and European Food and Safety Authority. THQ values of six heavy metals Cu, Zn, Cd, Cr, Pb and Fe were far below 1 in five species of the family Portunidae except Ni in *Scylla serrata* which was observed in high concentration.

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**ZOOPLANKTON PRODUCTIVITY AND DIVERSITY IN THE INDUS DELTA  
CREEKS DURING THE COASTAL SURVEYS OF 2013: PRELIMINARY  
OBSERVATIONS**

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The Indus delta creeks and the mangrove forest are traditionally known to be nurseries, with a major chunk of the fishing stock off the Sindh coast and shelf originating here. A number of studies report the presence of commercial species from the Indus delta creeks, but limited information is available linking the trophic levels to the fish stock or the presence of planktonic young stages of commercially important fishery components (fish, shellfish). As part of the Fishery Resource Appraisal Program of Pakistan (FRAPP, MFD), a major national fishery assessment exercise ongoing since 2009, in 2013, a coastal component was introduced. The aim is to assess the linkage of the fishery to the Indus delta creek's productivity. The National Institute of

Oceanography has been partners in this initiative, and for the Creek Survey Program (CSP) it is participating to provide the oceanographic support, including the plankton assessment from the creeks. This component is ancillary information to the bigger objective of the CSP project. This study presents the preparation exercise and shares the preliminary data findings with an overview of the zooplankton productivity and diversity of the selected creeks from August to December 2013. The zooplankton data set comprises of two component, mesozooplankton (335 µm) and ichthyoplankton (1000 µm) sampling. Both components are presented in here.

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**LABORATORY REARED DEVELOPMENTAL STAGES OF *LYSMATA VITTATA* (STIMPSON, 1860) (CRUSTACEA: DECAPODA: HIPPOLYTIDAE)**

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An ovigerous female of *Lysmata vittata* (Stimpson, 1860) was collected from Buleji, Karachi on 29<sup>th</sup> December, 2008. Larvae were hatched on 09<sup>th</sup> January, 2009, at room temperature 24°C, water temperature 23°C, in filtered sea water of a salinity of 35ppt and pH 7.4. Zoea stage I to VIII are illustrated, described and compared with the earlier studied larvae of same species and its congeners in the paper.

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**SPATIAL AND TEMPORAL DISTRIBUTION OF PENAEID SHRIMPS FROM THE SONMIANI BAY LAGOON, BALOCHISTAN, PAKISTAN**

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The one year investigation on spatial and temporal variation in the abundance of penaeid shrimps showed that *Penaeus indicus*, *Penaeus merguensis* and *Metapenaeus affinis* was dominant species in the Sonmiani Bay Lagoon, Balochistan. The influence of abiotic factors, water temperature and salinity on shrimps distribution was also evaluated. Shrimps were caught monthly at two sites (mouth area of the lagoon and mangrove creeks) within the Sonmiani Bay, Lagoon. A total of 1308 adult specimens were caught by gill net (near the mouth of lagoon) and 1247 juvenile specimens were caught by beach seine (from the mangrove creeks), with *P. indicus* being the dominant species. Seasonal variations were showed significant difference within species. *P. indicus* being more

abundant in SW monsoon and *M. affinis* in the post monsoon from the mouth area of lagoon and *P. indicus* were abundant in NE monsoon and *M. affinis* in SW monsoon from the mangrove creek. Abiotic factors, temperature and salinity seemed to be important variables in defining spatial and temporal distribution of penaeid shrimps.

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#### **DISTRIBUTION AND ABUNDANCE OF MARINE SPONGES AND ITS ASSOCIATED COMMUNITIES**

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Mangroves are salt tolerant coastal vegetation and most productive ecosystem of the world. They create distinctive ecological environment and support high diversity of flora and fauna (micro & macro-fauna). Sponges are sessile, simplest, multicellular, soft, encrusting and bottom dwelling animals that can survive in various aquatic environment. The present study was design to evaluate the seasonal distribution of sponge and their associated communities with respect to changes in physicochemical parameters at Sandspit backwaters. Growth were observed with relation to physicochemical parameters (dissolved oxygen, temperature, salinity, nutrients (NO<sub>3</sub>, NO<sub>2</sub>, NH<sub>3</sub> and PO<sub>4</sub> ions) and Chlorophyll). Sponge samples were collected using 1 m<sup>2</sup> quadrat and preserved in 90% ethanol. Between April to August (summer) sponges are highly abundant as compared to November to March (winter). Growth was abundant with increase of temperature during summer. Sponges provide food and shelter for wide variety of organisms through continuous flow of water and suspended organic matter. Different group of associated communities were identified including cyanobacteria, diatoms, isopods, copepods, amphipods, nematodes and other larval form of organisms. Water temperature ranging 21°C -32°C, where as salinity and pH range was 35-42‰ and 6.0 to 7.6, respectively. Nutrients were high in summer compare to winter.

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#### **DISTRIBUTION AND ZONATION PATTERNS OF PROSOBRANCH GASTROPOD *CERITHIDEA CINGULATUS* IN MANGROVE STANDS AT SANDSPIT BACKWATER**

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Mangroves create distinctive ecological environments that are characterized by a significant biodiversity. They provide food and shelter to a variety of organisms. Gastropods are the large and most diverse class of mollusc. To assess the seasonal

variation in biomass, distribution and abundance, of *C. cingulatus* sampling was performed in mangrove stands, at Sandspit backwater. A total of 14 core samples were collected quarterly from rhizosphere (7 cores) and non-rhizosphere (7 cores). The top 10 cm of each core was sliced at 1 cm and each layer was sieved through 1 mm<sup>2</sup> mesh. Live *C. cingulatus* were sorted, counted and weighted (wet). For sediment analysis 1 cm layers upto 10 cm of each core were used for the determination of water content, organic and inorganic matter and chlorophyll (chlorophyll *a*, *b* and total chlorophyll). Seasonal variation was observed in total number of *C. cingulatus* and the density also varies between two sites. The non-rhizosphere found most productive in term of biomass and number of *C. cingulatus*. During the period between January to April *C. cingulatus* are more abundant. The number ranges from 185-214 individuals/m<sup>2</sup> and 115- 119 individuals/m<sup>2</sup> in non rhizosphere and rhizosphere, respectively. Pearson correlation showed that *C. cingulatus* number were positively related to the concentration of total chlorophyll in the sediment and are not regulated by the distribution of sedimentary organic matter (negative correlation). The data will help understand the ecological niches that *C. cingulatus* would prefer in mangroves.

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#### 4. PALAEONTOLOGY

##### NEW FOSSILS OF *GAINDATHERIUM* (RHINOCEROTIDAE, MAMMALIA) FROM THE MIDDLE MIOCENE OF PAKISTAN

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New isolated teeth, maxillary and mandibular fragments from the Chinji Formation of the Lower Siwaliks are described and determined as *Gaındatherium browni* and *Gaındatherium vidali*. This material comes from the middle Miocene of Lava and Dhok Bun Ameer Khatoon localities, northern Pakistan. The fossil Remains recognized as *G. browni* are very similar to those previously known from the same formation in other Pakistani sites. Specimens from Lava site determined as *G. vidali* present morphological differences with respect to those of *G. browni*, being similar to those of *G. vidali* from the Nagri Formation, showing a greater size. Previously, *G. vidali* was reported only from the Nagri Formation of the Middle Siwaliks and the new material thus significantly widens the chronological distribution of this species in the continental deposits of the Siwaliks. This record implies that both species are not successive but coeval during the late middle Miocene.

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##### FIRST REPORT OF '*PROCOELODONTA*' (*PASALARHINUS*) *TEKKAYAI* FROM MIOCENE OF PAKISTAN

AMTUR RAFEH, MUHAMMAD AKHTAR, ABDUL MAJID KHAN, MUHAMMAD  
AKBAR KHAN AND RANA MANZOOR AHMAD

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In this paper, the new and unpublished rhinocerotid remains from the Lower and Middle Miocene from Siwaliks of Pakistan have been described and identified as '*Procoelodonta*' (*Pasalarhinus*) *tekkayai* previously known as (*Begertherium tekkayai*). These fossil remains have been collected from Chinji and Dhok Pathan formations of the Siwaliks of Pakistan. The newly discovered material includes isolated lower dentition. The taxonomic identification up to species levels is based on the morpho-metric measurements and morphological similarities of described specimens with published records of the species from Pasalar locality of Turkey. Previously, Pasalar was the only

locality where this species had been reported. The morphology of dentition indicates this species as a grazer.

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**DENTAL MORPHOLOGICAL STUDY OF *TRAGOPORTAX PUNJABICUS*  
FROM THE DHOK PATHAN FORMATION OF PAKISTAN**

RANA MANZOOR AHMAD, MUHAMMAD AKHTAR, ABDUL MAJID KHAN,  
MUHAMMAD AKBAR KHAN AND MUHAMMAD AMEEN

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The dental remains of *Tragoportax punjabicus* from the Dhok Pathan Formation (Middle Siwaliks) of district Chakwal, Punjab, Pakistan has been described to establish the taxonomic position of the newly discovered specimens. The identification of the upper and lower dentition is based upon the morphometric comparison with known material of this species. This study will add new specimens and information to the previously known database of this important Siwalik species regarding the dental morphology and taxonomy. The palaeo-environment of the Middle Siwaliks will also be discussed.

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**GAZELLA LYDEKKERI FROM DHOK BUN AMEER KHATOON, LOWER  
SIWALIKS OF PAKISTAN**

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Fossil site Dhok Bun Ameer Khatoon is an important locality of Lower Siwaliks of Pakistan. The fauna of DBAK mainly consists of Artiodactyla (suids, tragulids, giraffids, cervids and bovids) and Perissodactyla (Rhinocerotids). We have selected an important genus *Gazella* belonging to family Bovidae for thorough investigation. Eleven specimens including lower mandibles and isolated upper and lower molars have been collected and identified as *Gazella lydekkeri* and different aspect like evolution, taxonomy and biogeographic distribution have been discussed in detail which will be used to develop a baseline for the further investigations to support paleontologists in their future research. Previously its distribution was reported from the Dhok Pathan stage of the Middle Siwaliks, the Late Pliocene and Chinji type locality, Lower Siwaliks but we have

collected it from the locality Dhok Bun Ameer Khatoon, Chakwal District, Punjab, Pakistan ascribing it Middle Miocene age conferring to this discovery.

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**SOME NEW ARTIODACTYL REMAINS FROM THE MIDDLE SIWALIKS OF HASNOT (LATE MIOCENE), PAKISTAN**

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Newly discovered Late Miocene – Early Pliocene artiodactyl remains from the Middle Siwaliks of Hasnot (7.0-5.0 Ma), northern Pakistan is described and discussed. The Hasnot outcrops present various artiodactyl species including bovids, suoids, tragulids, and giraffids. The bovids, suoids and tragulids dominate the Hasnot artiodactyl assemblage. The bovids are represented predominantly by boselaphines and the tragulids by *Dorcatherium*. The artiodactyl community structure of the Hasnot Late Miocene – Early Pliocene (7.0-5.0) Ma is suggestive for establishing a vast open land environment depicting sporadic dry and flood seasons forcing a mosaic of ecotonal habitats with countless number of niches and parallel adaptations. The assemblages projected a mosaic of both more open and forested landscapes which comprised of heavy cover and, to some extent less significantly, forested, and wetlands.

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**NEW HIPPARIONINE FOSSILS (PERISSODACTYLA, EQUIDAE) FROM DHOK PATHAN FORMATION OF SIWALIKS, NORTHERN PAKISTAN**

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The new discovered fossil material represents the hipparionine genus *Sivalhippus*. The sample is excavated from the Late Miocene deposits of the Dhok Pathan type locality, situated in the Chakwal district, northern Pakistan. The described material consists of isolated dentitions and shows all the morphological features of *Sivalhippus*. These specimens provide new data and contribute to recent work from the Middle Siwalik outcrops of Pakistan. *Sivalhippus* is found abundantly in the Middle Siwaliks along with other Late Miocene mammalian groups. The studied hipparionine refers an

age of the Late Miocene-Early Pliocene for the fossiliferous deposits of the Dhok Pathan type locality.

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**PROBOSCIDEANS (VERTEBRATA: MAMMALIA) FROM DHOK PATHAN FORMATION OF SIWALIKS, NORTHERN PAKISTAN**

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Some new remains of proboscideans from the Late Miocene of the Siwaliks, northern Pakistan, were recovered and discussed. The represented proboscideans in the Dhok Pathan Formation of the Middle Siwaliks, northern Pakistan are normally medium sized. The material comprises isolated teeth, and mandibular and maxillar fragments. The newly discovered material can be referred to the Late Miocene proboscidean species found in the Dhok Pathan Formation of Pakistan. The recovered proboscideans indicate a mixture of wetland and woodland biomes in the Dhok Pathan Formation of the Siwaliks, and a suggestive of some open conditions during the time of deposition of the Dhok Pathan Formation in Late Miocene – Early Pliocene.

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**MERYCOPOTAMUS MEDIOXIMUS (ANTHRACOTHERIIDAE, SUIFORMES, ARTIODACTYLA) FROM MIDDLE SIWALIKS OF HASNOT, PAKISTAN**

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In this paper new fossilized cheek teeth of an extinct species of family Anthracotheriidae collected from Hasnot, district Chakwal, Punjab, Pakistan has been described. This sample includes maxillary fragment with premolar and molars and lower molar of the genus *Merycopotamus*. The taxonomic identification up to species levels is based on the morpho-metric measurements and morphological similarities of described specimens. These specimens are described under the genus *Merycopotamus* due to the presence of the diagnostic features of the genus in these specimens. Moreover, the mesostyle on upper molar is also a distinctive feature that helped the inclusion of these specimens into *Merycopotamus medioximus*. *Merycopotamus* is an extinct genus with



four species, *M. nanus*, *M. pusillus*, *M. dissimilis* and *M. medioximus*. This species *Merycopotamus medioximus* displays an intermediate morphology between *M. pusillus* and the more recent *M. dissimilis*.

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**RUMINANT FOSSILS (MAMMALIA, ARTIODACTYLA) FROM DHOK  
PATHAN FORMATION OF MIDDLE SIWALIKS, NORTHERN PAKISTAN**

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Some new fossils of the Late Miocene ruminants are documented from the Dhok Pathan Formation of northern Pakistan. The fossil record indicates the occurrence of three families of ruminants namely Bovidae, Tragulidae and Giraffidae in the Late Miocene – Early Pliocene outcrops of the Middle Siwaliks. The family Bovidae is the most predominant, quantitatively. The Late Miocene – Early Pliocene ruminants represent strong relationship with the Late Miocene – Early Pliocene sites of Greco-Iranian and Eurasian provinces. Regarding palaeoenvironmental interpretations, the taxa of these families indicate a predominance of woodland to savannah habitat during deposition of the Late Miocene – Early Pliocene.

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**SOME NEW REMAINS OF SUIDAE (ARTIODACTYLA, SUIFORMES) FROM  
DHOK PATHAN FORMATION OF SIWALIKS, NORTHERN PAKISTAN**

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New suid fossils were found in the Late Miocene deposits of the Siwaliks from the Dhok Pathan type locality, Chakwal districts, northern Pakistan. The material consists of left mandible fragments and isolated dentitions, and belongs to the genera *Tetraconodon* and *Propotamochoerus*. These specimens provide interesting information about the molars and incisors of the pig species, and throw light on the systematic position of these species. Biogeographically, the Late Miocene suids indicate strong relationships with Eurasian and African Late Miocene sites. The paleoenvironmental interpretations indicate a predominance of woodland to savannah habitat during the deposition of the Dhok Pathan Formation in the Pakistani Siwaliks.

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**SOME NEW FOSSILS OF *GAZELLA* (ARTIODACTYLA, RUMINANTIA, BOVIDAE) FROM DHOK PATHAN FORMATION OF SIWALIKS, NORTHERN PAKISTAN**

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New fossil gazelle, excavated from the Dhok Pathan type locality, Chakwal district of northern Pakistan, are identified, described and discussed here. The sample includes mandible fragments and isolated dentition. The specimens are identified as *Gazella lydekkeri*, a Late Miocene antelope. The new material extends our understanding regarding the Late Miocene antelopes. The sediments of the Dhok Pathan type locality date Late Miocene to Early Pliocene in age. The presence of the medium sized antelope in the Pakistani Middle Siwaliks indicates herbaceous vegetation during the Late Miocene – Early Pliocene.

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## 5. WILDLIFE, DIVERSITY AND CONSERVATION

### MORPHOMETRY OF SEROTINE BATS *EPTESICUS SEROTINUS* (SCHREBER, 1774) OF MALAKAND DIVISION, PAKISTAN

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The genus *Eptesicus* comprises 32 species. Geographically Serotine bats distributed in Australia; Africa; Eurasia; South, Central and North America. There are six species present in the Indian Subcontinent. The species has been reported from a single place Karakar Pass in Khyber Pakhtunkhwa-Pakistan. Fifteen bats were captured through mist net in Astanadaro Kalay (N34° 24.913' E71° 49.466'), Amluk Dara (N34° 43.773' E71° 52.502'), Barcharai Daim (N34° 33.737' E71° 44.872'), Bakrai (N34° 23.939' E71° 52.202'), Head Koper (N34° 24.454' E71° 50.061') and Pull Saokai (N34° 38.553' E72° 01.749') in Malakand division during the study. The mean body mass was 22.23g±4.18 (SD). Their head and body length was 69.80 mm±5.10 (SD) while their ear was 19.65 mm±0.96 (SD) long. The mean greatest length of the skull was 21.40 mm±0.70 (SD). The breadth of braincase was 9.69 mm±0.22 (SD) while that of post-orbital constriction was 4.72 mm±0.14 (SD) long. The total baculum length of seven specimens was 1.00 mm±0.15 (SD). The collected data was analyzed to investigate morphometric differences in the population of the Serotine bats in Malakand Division with other already mentioned in literature and document new distribution map of this species in Pakistan.

### SEASONAL DIET COMPOSITION OF COMMON LEOPARD (*PANTHERA PARDUS*) IN MACHIARA NATIONAL PARK, AJ&K, PAKISTAN

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Machiara National Park is one of the suitable habitats for large variety of wildlife including leopard in Pakistan, which constitutes a broad range of prey species like wild animals and livestock. The present study was designed to assess the type of prey species used by leopard in winter and summer seasons, by their scat analysis. The hair

mounting technique was used to identify species consumed by leopard. Furthermore habitat analysis, predator-prey relationship and human-carnivores conflict were also assessed. The scat analyses revealed that the most frequently consumed prey species were Grey goral (18.08%) and Indian pika (13.45%) in winter, summer respectively. Moreover Musk deer, Cape hare, Monkeys, Royle's mountain vole, Kashmir marmot, Stoat or Ermine, Musk rat, House mouse, Flying squirrel, Himalyan palm civet, House rat, Sheep, Goat, Cow, Horse, Red fox, Birds and some unknown were collectively constitutes 22.59% and 16.16% of leopard food in in summer and winter respectively.

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#### **CO-EXISTENCE OF LARGE CARNIVORES AND HUMANS IN MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN**

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The present study was conducted to highlight the co-existence of large carnivores and humans in terms of livestock depredation, pattern of predation, human causality and perception of people toward large carnivores in remote areas of Azad Jammu and Kashmir, Pakistan. The study was conducted in 30 villages of three Union Councils by semi-structured interviews base, and the research reports related to the large carnivores of the area. The average populations of goat ( $7.24 \pm 7.081$ ) sheep ( $5.85 \pm 4.06$ ), cow/ox ( $1.79 \pm 2.33$ ), buffalo ( $0.67 \pm 1.73$ ), horses/donkey ( $1.21 \pm 2.21$ ), dog ( $1.09 \pm 1.02$ ), Chicken ( $6.95 \pm 4.00$ ) were owned by the local peoples. Total average livestock losses due to theft, disease, Common leopard, Black bear and Asiatic jackal were  $N= 0.55$ ,  $N= 5.39$ ,  $N= 4.09$ ,  $N= 0.53$ , and  $N= 0.36$  respectively. Livestock grazing and human involvement into the carnivores habitat, poor agro-grazing practice and poor husbandry are the main cause of the human carnivores conflict. Education about the conservation, monitoring of the large carnivores and dog guards along with livestock head may help to reduce the human-carnivores conflict.

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#### **ASSESSMENT OF AMPHIBIAN FAUNA AND THEIR ENVIRONMENT THROUGH PHYSICO-CHEMICAL PARAMETERS IN DISTRICT JAMSHORO, SINDH PAKISTAN**

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Amphibians have gained importance in economic sectors for their agricultural, food and medicinal value therefore their habitat is being analyzed by several international

wildlife organizations because of their decline in various parts of world due to chemical contamination and water pollution. In order to contribute to this global issue, present study was carried out in District Jamshoro surveyed monthly during 2012 for the determination of amphibian diversity and water sampling from their habitat. Amphibian species were identified by using morphometric and taxonomic key, while environment was analysed through Physico-chemical parameters viz: pH, Conductivity, Total dissolved solids (TDS), Hardness, Alkalinity, Sulfate, Phosphate, Nitrite, Nitrate and Carbon dioxide. During this study three species of family Ranidae viz: *Hoplobatrachus tigerinus* (Genus *Hoplobatrachus*), *Euphlyctis cyanophlyctis*, (Genus *Euphlyctis*) *Allopa hazarensis* (Genus *Allopa*) and one species of Family Bufonidae: *Bufo stomaticus* (Genus *Bufo*) were identified to exist in study area. Physico-chemical analysis indicated high level of few parameters i.e. Conductivity, TDS, Hardness, Sulfate, Phosphate and Nitrite, which may affect amphibian survival.

#### **STUDY OF DEFORMATION OF INCISOR TEETH IN FAMILY MURIDAE (RODENTS) SINDH, PAKISTAN**

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Malocclusion is malposition of the teeth that prevents alignment of the teeth in jaws, making rodents unable to chew hard food and developing sour in mouth parts. In order to examine the population of rodents affected with Malocclusion, District Dadu, Larkana and Jacobabad of Sindh were surveyed from 2006-2009. Only 10 individuals were recorded to be affected with malocclusion out of 127 collected and examined individuals of Muridae family.

#### **THE ROOST CHARACTERISTICS AND MORPHOLOGY OF THE NAKED RUMPED TOMB BAT (*TAPHOZOUS NUDIVENTRIS* CRETZSCHMER, 1830) IN FAISALABAD DISTRICT**

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The naked rumped tomb bat *Taphozous nudiventris* Cretzschmer, 1830 is extensively distributed in Pakistan. One colony of these bats was recorded from the study area. The naked-rumped tomb bats (*Taphozous nudiventris*) (Emballonuridae: Chiroptera) has never been reported from Faisalabad district. This study was carried out

from June, 2012 to December, 2012 to study the roost characteristics and morphology of the Naked Rumped Tomb Bat (*Taphozous nudiventris* Cretzschmer, 1830) in Faisalabad district. In the present study *Taphozous nudiventris* was captured from the building of Hussain Sugar Mill situated on main road from Faisalabad to Lahore Tehsil Jaranwala having co-ordinates 31°19'48.3" North, 73°24'12" East and is 477 feet above the sea level. We captured twenty two bats through hand net from the study area. Of these two were male and twenty were female. Body mass, external body, cranial measurements and bacular measurements were recorded of while five bats were preserved at bat lab in the department of Zoology and Fisheries University of Agriculture Faisalabad.

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**POPULATION STATUS OF CHUKAR (*ALECTORIS CHUKAR*) IN  
UTMANKHEL, BAJAUR AGENCY, PAKISTAN**

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The chukar (*Alectoris chukar*) is the national bird of Pakistan and a Eurasian upland gamebird in the pheasant family Phasianidae. The study was conducted to assess population density of *Alectoris chukar* in Uthmankhel Bajaur Agency (study area 129 km<sup>2</sup>) from March to August 2013. Four study sites Sapary, Salosar, Godar and Garay were selected from the study area. Each site was visited on fortnight bases for data collection. Surveys were schedule early in the morning and late evening. Population density was estimated by using Point Count Method. Mean population density was observed at Sapary about (0.00002±0.000005) / 50m<sup>2</sup>, at Salosar (0.00001±0.000002) / 50m<sup>2</sup>, at Godar (0.00002±0.000008) / 50m<sup>2</sup> and at Garay (0.00002±0.000000) / 500m<sup>2</sup>. Analysis of variance showed, that no significant difference (p>0.05) was observed in population density / 50m<sup>2</sup> in four different sites. At Sapary in mean morning population density (0.00002±0.000004) / 50m<sup>2</sup>, at Salosar (0.00002±0.000003) / 50m<sup>2</sup>, at Godar (0.00001±0.000003) / 50m<sup>2</sup> and at Garay (0.00002±0.000002) / 50m<sup>2</sup>, while mean population density in evening at Sapary (0.00002±0.000006) / 50m<sup>2</sup>, at Salosar (0.00002±0.000002) / 50m<sup>2</sup>, at Godar (0.00002±0.000003) / 50m<sup>2</sup> and at Garay (0.00002±0.000002) / 50m<sup>2</sup> were noted. Through paired t-test no significant difference (p>0.05) was observed between morning and evening mean population density / 50m<sup>2</sup>. Total population of *Alectoris chukar* in 129 km<sup>2</sup> of Uthmankhel Bajaur Agency about 53.922 at morning time and about 46.956 individuals were recorded at evening time. The study provides the first scientific record about the important ecological aspect of population density of the national bird in Uthmankhel Bajaur Agency.

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### RECENT RECORD OF *SCOTOPHILUS HEATHII* FROM WHEAT-RICE BASED AGROECOSYSTEM OF PUNJAB

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Bats are the second most diverse group of mammals in Pakistan, but there is extreme shortage of data regarding their occurrence and distribution in the country. The focus of the present study is to explore Asiatic Greater Yellow House bat *Scotophilus heathii* from central Punjab from where the species has not been documented yet. The study area comprised three districts namely Gujranwala, Hafizabad and Mandi Bhaudhin which are main rice producing districts in the country. The bat species were captured from January 2011 through December 2012 and a total of 33 *S. heathii* were captured from the study area. The external body, cranial and bacular measurements of the captured specimens were compared with available literature. The cranial and bacular features of the species which are important traits for mammalian identification are reported for the first time in country.

### FIRST RECORD OF BLYTH'S HORSESHOE BAT, *RHINOLOPUS LEPIDUS* FROM PUNJAB, PAKISTAN

ARSHAD JAVID<sup>1</sup>, MUHAMMAD SHAHBAZ CHATTHA<sup>1</sup>, MUHAMMAD MAHMOOD-UL-HASSAN<sup>2</sup>, SYED MAKHDOOM HUSSAIN<sup>3</sup>, SANA ASHRAF<sup>1</sup> AND MUHAMMAD IDNAN<sup>1</sup>

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*Rhinolophus lepidus* is an endemic bat of south Asian region but the status of the species from its native territory is still unknown. From territorial limits of Pakistan, single specimen of the species was captured in 1985 from Abbotabad, Khyber Pakhtunkhwa province. During present study three districts namely Gujranwala, Hafizabad and Mandi Bhaudhin that are known for rice-wheat production in the country were surveyed to explore bat fauna of the region. A bat roost containing 70 *R. lepidus* was found at Rasul

Nagar, district Gujranwala from an underground cellar and out of these, 10 (4♂, 6♀) were captured with the help of a hand net. The average head and body length of all the captured specimens was  $42.36 \pm 1.1$  mm, forearm length was  $40.34 \pm 0.75$  mm, greatest skull length (n= 2) was  $17.5 \pm 0.49$  mm while baculum (n= 2) was  $4.3 \pm 0.38$  mm long. The present record is first report from the study area and the Punjab province.

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### AGE AND SEX SPECIFIC SEASONAL CHANGES IN THE WEIGHT OF ASIATIC BLACK BEARS (*URSUS THIBETANUS*) IN BALKASAR BEAR SANCTUARY

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Seasonal changes in weight have been studied by comparing growth curves fitted to sex-age-weight data of Asiatic black bears (*Ursus thibetanus*) obtained from Balkasar Bear Sanctuary. The data for the weight were obtained from bears (n=10) during 2008-10. The 10 bears were categorized into three age groups. Group A; with cubs of a year continually adds up in kilos irrespective of the season. While in group B; of young male and female bears of 7-8 years show increase in weight during summer season, while there was around 20 Kg decrease in weight of female bears during winter. The bears of group C, mature bears aged 13- 15 years put on during summer and loose in winter. The seasonal fluctuations in weight are more obvious in females compared to males in group B and C. Behavioral and physiological changes associated with changing seasons explained weight fluctuation in bears of different ages and sexes. The study generated baseline data for the conservationists working in the field.

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### OUTBREAKS OF NEW CASTLE DISEASE (ND) IN PEACOCKS OF KHYBER PAKHTOONKHA PROVINCE OF PAKISTAN DURING THE YEARS 2011-12

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The huge outbreak of Newcastle disease (ND) during August and September in 2011 and 2012 in the Wildlife conservation park in Bannu, KP was analyzed in the peacock population. Newcastle disease is a fatal, highly contagious viral disease of domestic and wild avian species. Its global impact is enormous and unsurpassed by any other poultry disease. ND is caused by Newcastle disease virus (NDV) which is classified as an Avulavirus in the family Paramyxoviridae. The outbreak was more in adults as well



as in young chicks of 5 to 11 months of age. The vaccination schedule in 2011 includes NDV vaccine while in 2012 NDV, NDV lasota and a combination of ND and IB vaccine were used. The mortality rate in adults in 2011 and 2012 was 70% and 71.19%, while in chicks was 70.37% and 72.8%, respectively. The mortality rate in chicks in 2011 was in the decreasing order of 100%, 79.16%, 76.67%, 76.19%, 51.06%, in cameo, Blue, Pied, Black shoulder and White breeds, respectively, while the mortality rate in 2012 in chicks was in the decreasing order of 100%, 84.62%, 68.18%, 64.71% and 63.16% in Black shoulder, Cameo, Blue, White and Pied breeds. This high mortality rate may be due to their lower immune system or maybe due to the new strains of virus, or vaccine failures or less expert availability.

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#### **HUMAN DIMENSION OF THE CONFLICT AND RETALIATORY KILLING OF COMMON LEOPARD (*PANTHERA PARDUS*) IN AZAD JAMMU AND KASHMIR**

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The common leopard (*Panthera pardus*) is critically endangered to Pakistan. Except a few thick forest patches and protected areas, where a reasonable degree of protection has helped the species to grow in numbers, it is dwindling in other parts of its distribution range and is on the verge of extinction. Leopard killings by humans have been recorded from different areas in the past and it is the most serious threat to the leopard population in Azad Jammu and Kashmir (AJ&K). Illegal killing of leopard has grown in recent years because of the prey paucity and the steady buildup of people and livestock on the forest fringes. AJ&K is one of the high risk areas for leopards as they were continuously being killed at different locations for last decade. The data were collected on leopard killings in AJ&K from year 2000 to 2013, revealed about 139 leopards have been killed in past 13 years. Maximum killings were reported in 2012 and majority is during the summer season. The adults were killed relatively in higher number, outside the protected areas and along the LOC. Maximum killings were in response to the livestock depredation by leopard. The high rate of killing is serious threats to the survival of species. As a result of increasing biotic pressures leopard has become rare with growing threat of further degradation and fragmentation of its habitat. If situation continue, in the future it may be extinct regionally and will result in negative imbalance for our food chain. Sustainable and culturally acceptable conservation solutions are required for reducing the threats to leopard and lowering the losses by local communities in AJ&K.

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### QUANTIFYING THE RANGE REDUCTION OF THE SUNDA CLOUDED LEOPARD (*NEOFELIS DIARDI*) IN TROPICAL ASIA

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The Sunda clouded leopard (*Neofelis diardi*) is a medium-sized wild cat species, which is one of the two species of clouded leopard recently separated on the basis of genetic analysis. Its distribution is restricted to the islands of Sumatra and Borneo in the world. In the current study, we investigated and quantified the historical and current distribution ranges of this species. Data about historical distribution were collected from all forms of published literature while spatial data for its current distribution were obtained from IUCN Red List of Threatened species 2008. The quantification and analysis of distribution ranges were carried out by using Quantum Geographical Information system (QGIS). Results have shown that sunda clouded leopard occupied an area approximating  $9.777 \times 10^5$  km<sup>2</sup> in history while at present it occupies an area approximately  $4.935 \times 10^5$  km<sup>2</sup>, showing 49.52% range reduction. The numbers as well as size of the protected areas that contained sunda clouded leopard in history and at current have also been reduced by 64.91% and 27.63%, respectively.

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### PROTECTED AREA ZONATION USING GEOSPATIAL TECHNOLOGIES: A CASE STUDY OF MACHIARA NATIONAL PARK, AJK, PAKISTAN

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Machiara National Park (MNP) lies in the District Muzaffarabad, Azad Jammu & Kashmir, Pakistan and is a representative of the unique biodiversity of Western Himalayan Ecoregion. The area of the Park is highly diverse in biological and physical characters. Protected Areas has always been under stress by the human activities living in and around these areas. Machiara National Park is also under threat by illegal and

uncontrolled forest cutting, natural and man-made damage to natural resources, uncontrolled grazing, unsustainable extraction of medicinal plants, encroachment and land use change due to unclear boundaries, lack of data on forest resources etc. This is due to the population residing adjacent to the park boundary which is directly or indirectly dependent on the natural resources of the Park. A possible way to deal with the conflicting demands on a protected area is zoning, which considers the overall management objectives for a park and sets aside designated areas that permit or give priority to certain activities. Considering the ecological, geographical and socioeconomic aspects the ecological zoning of Machiara National Park was carried out. Land cover mapping, thematic layers development and habitat mapping of key fauna species was carried out using ground truth data, SPOT 5 satellite images, Survey of Pakistan topographic maps, which helped in delineating the different zones of the park. The suitability of areas for the conservation of key species, local community needs and visitor activities were also considered in zoning activity. Ecological zoning includes a Conservation Zone or Core Zone (special preservation area), Buffer and Sustainable Use Zone, (conservation and multiple use area), Recreation Zone and Sustainable Development Zone. This zoning system classified the different areas according to their need for protection and sustainable development. The study can be considered as an effective step towards management and conflict resolution of Protected Areas worldwide.

**HABITAT USE AND POPULATION OF KALIJ PHEASANT (*LOPHURA LEUCOMELANOS*) IN SANGHER BATTARA FOREST, DISTRICT BAGH, AJ&K**

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White-crested Kalij pheasant (*Lophura leucomelanos hamiltoni*) is the only pheasant species found in Pakistan. Present study was conducted (from August 2012 to July 2013) to assess the population status and explore habitat preference of white-crested Kalij pheasant in Sanghar Bathara forest area of Azad Jammu and Kashmir (AJ&K). The forest area was predominated by three forest types viz moist temperate forest, sub-tropical pine forest and sub-tropical semi-evergreen forest. Line transect method was employed to record direct and indirect evidences of the white-crested Kalij pheasant. The vegetation sampling revealed that floral composition of the moist temperate forest dominated by *Pinus wallichiana*, and *Quercus incana*. The Kalij pheasant habitat in subtropical pine forest was dominated by *Pinus roxburghii* and *Olea ferruginea* while the dominated

vegetation species in subtropical semi evergreen forest were *Olea ferruginea*, *Quercus incana* and *Pinus roxburghii*. In total 118 birds were directly sighted during the course of this study over an area of 20 km<sup>2</sup>, which has resulted in to population estimates of 5.9 birds/km<sup>2</sup> in the forest ecology of Sanhgar Bathara. The adult birds sighted lead to calculate a sex-ratio of 0.98 cocks to 1 hen. In moist temperate forest 4.2 birds/ km<sup>2</sup> were sighted with a mean encounter rate of 0.84 birds per line transect. In sub-tropical pine forest only 2.5 birds/ km<sup>2</sup> were sighted. While the highest density of Kalij was 11 birds/km<sup>2</sup> was observed in semi evergreen scrub forest and southerly distributed.

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### **HABITAT CHARACTERISTICS AND DISTRIBUTION OF ASIATIC BLACK BEAR (*URSUS TIBETANUS*) IN MACHIARA NATIONAL PARK, AJ&K**

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Asiatic black bear (*Ursus thibetanus*) belongs to family Ursidae and is found in Khyber Pakhtoonkhwa (KPK), Gilgit Baltistan (GB) and Azad Jammu and Kashmir (AJ&K) in Pakistan. It inhabits dense Himalayan moist temperate forest, Himalayan dry temperate forest and tropical pine forest, and does not ascend above the permanent tree line into the alpine regions. Asiatic black bear is vulnerable globally as well as in Pakistan. The present study was conducted from September 2012 to May 2013 to determine the distribution range and habitat characteristics of Asiatic black bear in Machiara National Park, AJ&K. For this purpose, study area was divided in three sites *i.e.* Machiara, Bheri and Sarli Sacha and 45 transects were established covering an area of 7.8 km<sup>2</sup> for data collection. Distribution range was determined through direct observations as well as indirect evidences (scats, foot prints, claw marks, soil digs etc.) in its habitat using sign survey. For habitat characteristics, vegetation survey of study area was conducted in addition to noting physical features of its habitat. Human-bear conflicts were also assessed during the study. The Asiatic black bear is distributed in all the three selected sites of the park between altitudinal ranges of 1589m to 3259m. Distribution map of the black bear in the park was developed. Sign observed were high in Bheri (62%) as compared to Machiara (20%) and Sarli Sacha (18%). Forest areas contained maximum signs (90%) of the bear. A total of 63 plant species were recorded in black bear habitat. Dominant plants were *Pinus wallichiana*, *Abies pindrow*, *Aesculus indica*, *Prunus cornuta*, *Picea smithiana*, *Viburnum nervosum*, *Indigofera heterantha*, *Sobaria tomentosa*, *Adiantum incisum forsk*, *Cymbopogon martini*, *Dryopteris stewartii*, *Poa inferna*, *Persicaria nepalensis*, *Fragaria nubicola* and *Fragaria vesca*. Most sightings were recorded in the months of May (29%) and September (22%) during day time 33% (n=15). Kruskal-Wallis Test showed a significant difference in vegetation in three study sites. Maximum livestock depredation by black bear occur at Sarli Sacha (40%) followed by Machiara (35%) and Bheri (25%). Maximum livestock was depredated at young stage

(60%) in the forest area (65%). Out of total conflicts events, 68% were the crop damaged by the Asiatic black bear. The survey results showed that herding practices enhances disturbance to habitat of Asiatic black bear in the area which has resulted in to livestock losses and also resulted in crop damaging that in turn creates the negative perceptions of the local community about the bear.

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**SEASONAL VARIATION IN MALE GONADS OF SMALL INDIAN  
MONGOOSE (*HERPESTES JAVANICUS*) IN POTHWAR PLATEAU**

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Small Indian mongoose (*Herpestes javanicus*) is a diurnal carnivore and an active burrower. It occurs in southern Sindh, Baluchistan and Punjab provinces, in the Pothwar region, it occurs in Salt range, Jhelum, Rawalpindi and Attock districts. It is adapted to inhabit the villages bordering areas and towns. The species serves as a biological control for rodents and snakes in the agro-ecosystems. In the current study, male individuals of the animal species were live trapped from the study area from July 2012 to June 2013, on monthly basis and sacrificed to obtain blood and testicular tissue samples. Blood was centrifuged at 3500 rpm to obtain plasma for estimation of hormonal concentrations (testosterone, FSH and LH) using commercially available ELISA hormone kits. Tissue samples of the testes were processed for standard histological procedure whereby light microscopic sections (5 – 7  $\mu$ m thick) were stained with Hematoxylin and Eosin to observe different stages of spermatogenesis inside the seminiferous tubules viz. primary spermatocytes, secondary spermatocytes, and spermatids. Frequency of occurrence of spermatozoa inside the seminiferous tubules was determined using a grid while changes in seminiferous tubular diameter, and epithelial cell height were quantified using stage and ocular micrometers. Results showed, in general, higher concentrations of testosterone from January to March, and then from June to September, indicating that it breeds twice a year. The concentration of LH was also found higher during these months. Frequency of occurrence of spermatozoa in the seminiferous tubules differed non-significantly ( $p > 0.05$ ) but showed variation among individuals of active and inactive seasons; the average counts of the breeding season being higher compared to non-breeding season. Similarly, the size of epithelial cell height (initial, middle and terminal segments) differed non-significantly ( $p > 0.05$ ). A positive *Pearson correlation* ( $r$ ) was found between testes weight and LH concentration ( $r = 0.41$ ), testes weight and diameter of seminiferous tubules ( $r = 0.68$ ), testes weight and mean frequency of spermatozoa ( $r = 0.40$ ), and testosterone concentration and diameter of seminiferous tubules ( $r = 0.45$ ). The study concludes that breeding season of the small Indian mongoose extends from March till September in the study area while its non-breeding season ranges from October to next February.

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**BREEDING ECOLOGY OF SMALL INDIAN MONGOOSE (*HERPESTES JAVANICUS*) IN DISTRICT CHAKWAL, PAKISTAN**

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The current study investigated reproductive pattern of small Indian mongoose (*Herpestes javanicus*) in district Chakwal of Potohar Plateau from June 2012 to June 2013. Breeding season, reproductive behavior, sex ratio, length of gestation period, litter size and parental care of the animal species were studied at five selected sites. Data were recorded by visual observations as well as by live capture. Burrow occupancy was checked by establishing foot prints outside the burrow openings. Litter size was determined by three methods viz. by counting numbers of young ones accompanying parents, counting of developing embryos and counting of placental scars. Small Indian mongooses breed twice in a year. Out of 31 females captured, only five (16%) were found pregnant. The sex ratio of mongoose populations at selected sites was found to be 0.67 (n=52). Observations on mating behavior showed mongooses as polygynous and they share burrow during breeding season. Length of the gestation period was approximately 51 days. Mean litter size was  $2.83 \pm 0.14$  by visual observations,  $3 \pm 0.24$  by counting placental scars and  $3.2 \pm 0.37$  by counting of developing embryos. Field observations on parental care showed that females train their pups during hunting trips.

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**DISTRIBUTION AND HABITAT USE OF KALIJ PHEASANT (*LOPHURA LEUCOMELANOS*) IN MARGALLAH HILLS NATIONAL PARK, ISLAMABAD**

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White-crested Kalij pheasant (*Lophura leucomelanos hamiltonii*) is found in the Margallah Hills National Park (MHNP) and is threatened in Pakistan. Present study was conducted to determine its distribution range and habitat status in MHNP. Data on its distribution was collected through direct sightings of birds and noting the indirect signs such as fecal material, feathers and calls. Coordinates of occurrence points were recorded. For habitat status, vegetation survey was conducted by using quadrat method to estimate density, diversity and species richness in addition to noting other features of habitat such as aspect, altitude, distance from water source and human disturbance. Data on threats was collected through questionnaire survey of local people and wildlife staff. Kalij pheasant was found distributed in all four ranges of the park four ranges (Bharakau, Nurpur, Saidpur and Golra) between 615m to 1050m elevation. Its occurrence was more frequent in Saidpur Range and Nurpur range due to dense vegetation as a result of better conservation and presence of water sources. Kalij was mostly found on southern and

eastern southern slopes of the hills due to dense forest cover as compared to northern one. Overall, 61 plant species were identified in habitat of Kalij pheasant in MHNP which included 23 tree species, 18 shrubs, 7 grasses and 13 herbs. Most dominant plant species of Kalij pheasant habitat were *Carissa opaca*, *Dodonaea viscosa*, *Buxus papillosa*, *Cynodon dactylon*, *Cassia fistula*, *Myrsine africana*, *Justicia adhatoda*, *Zizyphus nummularia*, *Dalbergia sissoo*, *Olea ferruginea*, *Grewia optiva*, *Woodfordia fruticosa* and *Pinus roxburghii*. Student *t*-test showed no significance difference in means of relative densities, relative frequencies and importance value indices of plant species among the four ranges of the park. However, a significant difference in relative cover (*p*-value=0.850 which is more than 0.5) was found in Nurpur range with more relative cover. Major threats faced by Kalij pheasant in MHNP included poaching, deforestation, forest fires, live stock grazing activities, fuel wood cutting, encroachment and disturbance due to tourist activities.

#### **POPULATION DENSITY AND HABITAT PREFERENCE OF HEDGEHOG *HEMIECHINUS COLLARIS* IN DISTRICT ATTOCK**

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The study on population status and habitat preference of hedgehogs in district Attock of Pothwar plateau, Pakistan was carried out from September 2012 to August 2013. Eight study sites (villages) were selected and at each location three different habitats *i.e.* cultivated cropland habitat, non-cultivable wild habitat, and village houses were searched for direct and indirect signs of hedgehog species. Only Indian long eared hedgehog (*Hemiechinus collaris*) was recorded. In cropland habitat a total of 13 live animals were observed giving mean number of live hedgehogs ( $2.29 \pm 0.94$ ) per linear km. Similarly, 22 active burrows were recorded with mean prevalence of  $3.8 \pm 1.43$  burrows per linear km. High number of hedgehogs were seen in monsoon season while more active burrows were recorded in spring season. In wild habitat only 5 live animals were observed giving mean number hedgehogs ( $1.37 \pm 0.61$ ) per linear km. Whereas, 16 active burrows were recorded with mean prevalence  $2.63 \pm 0.9$  burrows per linear km. Unlike the cropland, relatively more live hedgehogs were seen in spring and summer seasons in the wild areas. However, equal distribution of active burrows was recorded during spring, summer and fall seasons. In both of these habitats, no sign of presence of hedgehog was recorded during the winter season, indicating complete hibernation. Vegetation analysis showed dominance of *Ziziphus muritiana* and *Acacia modesta* trees in both of these habitats. The dominant shrubs were *Prosopis juliflora* and *Ziziphus nummularia*. Whereas dominant herbs and grasses were *Cynodon dactylon*, *Tetrapogon tenellus*, *Calendula arvensis*, *Sanchus asper*, *Cymbopogon spp* and *Sorghum helepense* in both cultivated fields and wild habitats. In residential areas, about 31% houses were positive for any sign of the hedgehog. In total 19 hedgehogs were seen in 183 houses indicating a

population on 0.10 hedgehog per house or one hedgehog in 10 houses. Unlike field situation the hedgehog were seen active during months in the village houses. It shows that populations of hedgehog have adopted to live in human dwellings to avail frequent supply of food, year around shelter, stable climatic and environmental conditions and protection from predators.

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### **FEEDING PREFERENCE OF HOG DEER (*AXIS PORCINUS*) UNDER CAPTIVITY**

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Food is a basic and most important component for keeping a wild ungulate healthy in captivity. Wild animals are free roaming species in natural habitat where variety of natural floral species are consumed as integral part of their diet. It becomes important to assess the preferred diet combination for these species in captive conditions. This study determined feed preference of Hog deer in captivity, performed proximate analysis of preferred plants and plants collected from its natural habitat. Seven experimental animals were offered weighed amount of six types of feed including oat, alpha alpha, sorghum, maize, whole wheat grains and chick pea in three binary combinations *i.e.* C1: Alpha alpha and oat, C2: maize and sorghum; C3: chickpea and whole wheat grain. Eleven plant samples were collected from natural habitat of Hog deer in Tansa Barrage Wildlife Sanctuary based on information from local people and wildlife watchers. These plants and those offered in captivity were analyzed for proximate analysis at Animal Nutrition Laboratory of National Agricultural Research Centre, Islamabad for Crude protein, Fiber, Fat and Ash. Hog deer showed significant difference in consuming feed offered in binary combination having feed preference of  $93 \pm 2.38\%$  for oat,  $93 \pm 2.74\%$  for maize and  $95 \pm 3.94\%$  for chick pea by males and  $89.9 \pm 2.49$  for oat,  $91.2 \pm 3.77$  for maize and  $90.8 \pm 2.79$  for chick pea by females. In oat, crude protein, ash, fiber and fat contents were 6, 7, 4.5 and 25%, respectively, in maize 19, 23, 1.7 and 24%, respectively and in chick pea 23, 4, 4.5 and 11%, respectively. Oat, maize and chick pea were preferred feed of Hog deer in captivity which are rich in nutrients that are required for proper feeding. Hence, feed of captive hog deer could be improved by using oat with chick pea during winter and maize with chick pea during summer. Proximate analysis of preferred wild plants revealed that nutritional requirements of hog deer are directly related to the nutritional contents obtained. Degraded habitat of hog deer could be restored by proper protection and plantation of preferred plants for their feeding requirements. The present study can also help zoos, wildlife parks and safari parks to improve nutritional requirement for captive ungulates ultimately resulting in healthy population of captive animals especially the threatened species.

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### HABITAT USE AND POPULATION OF HEDGEHOG SPECIES IN TALAGANG AREA, DISTRICT CHAKWAL

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Four species of hedgehog *Hemiechinus auritus*, *Hemiechinus collaris*, *Paraechinus micropus* and *Paraechinus hypomelas* belonging to order Insectivora and family Erinaceidae present in Pakistan. The present study was conducted to study habitat use and population density of hedgehog in Tehsil Talagang of district Chakwal, Pakistan. Hedgehog habitat in cultivated land had dominant plant species such *Acacia modesta*, *Melia azedarach*, *Fagonea indica*, *Adhatoda zeylanica*, *Convulus arvensis* and *Carthamus oxycantha*. In natural forest habitat dominant plant species were *Prosopis cineraria*, *Zizyphus nummularia*, *Calotropis procera*, *Lantana camara*, *Adhatoda zeylanica* and *Gymnosporia royleana*. *Euphorbia helioscopi* and *Fumeria indica*. The hedgehog spends more time in shrubs and grasses as compared to trees because they provide them protection from predators. The fruits of *Zizyphus nummularia* were believed to be the favorite food of hedgehog. In human habitation area, dominant plant species were *Acacia modesta*, *Adhatoda zeylanica*, *Convululus arvensis* and *Taraxacum officinale*. The overall population density of hedgehog in human habitation was  $43 \pm 5$  animals per km<sup>2</sup> and  $40.66 \pm 7.5$ ,  $39.33 \pm 4.4$  animals per km<sup>2</sup> were recorded in cultivated area and natural habitat area, respectively. Seasonally, population density of  $16.66 \pm 0.3/\text{km}^2$  was recorded in summer,  $11.33 \pm 0.2/\text{km}^2$  in winter and  $10.66 \pm 0.2/\text{km}^2$  in spring.

### DISTRIBUTION AND POPULATION STATUS OF CANIS SPP. THREATS AND CONSERVATION IN LEHRI NATURE PARK, SALT RANGE, DISTRICT JHELM

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The Grey wolf has been ranked endangered and Asiatic Jackal as near threatened in Pakistan. Scientific data on population and threats to these species are not available in Pakistan, which is required for their proper management and conservation. The present

study was conducted to collect data on distribution range, population status and threats to both of these *Canis* species in Lehri Nature Park. The data was collected using direct observations and indirect signs in the field. The population of grey wolf and Asiatic Jackal were scattered into pocket of the study area and its surroundings. The current population of grey wolf was estimated 06 individuals and that of Asiatic jackal 28 individuals in the study area. The present study showed that Grey wolf and Asiatic jackal were distributed in the northern and southern part of the study area having dense vegetation cover of tress and shrub between the altitudes of 330 m and 515 m. The research finding revealed that the scrub forest is the most preferred habitat of both the species but due to anthropogenic pressure the scrub forest is under severe threat. The dominant trees species were *Acacia modesta*, *Zizyphus nummularia*, and *Prosopis juliflora* and shrubs species of *Dodonea- viscosa*, *Calotropis procera* and *Adhatoda vasica*. Urial is one of the natural prey species: their population is low due to a number of reasons and therefore the maximum dependence of the wolves was on the livestock of the local and nomadic shepherds. The main prey species in the livestock was goats and sheep. The interviews were conducted with the eye witnesses of wolf attacks including livestock were killed by 5-6 numbers of wolves in different hamlets in the study area. The killing rate of the livestock by the wolves was greater when the nomadic shepherds were present in the area and decreased when they left the area. Presence of nomadic shepherds and killing rate has relation with the shifting of the wolves from the study area. It is further concluded that the population of the Grey wolf and Asiatic jackal has decreased over the time due to less availability of the natural prey species and habitat destruction.

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#### BAT FAUNA OF ISLAMABAD, PAKISTAN

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The present study was conducted in Islamabad, the capital city of Pakistan to explore microchiropteran fauna of the city. A total of 3600 m<sup>2</sup>hrs of mist netting effort resulted in the capture of 72 bats representing two Families, four Genera and nine Species. The overall netting index was 1.64. Netting index for two *Taphozous* spp. was not calculated as these Embellonurids were captured from their roost with the help of hand nets. *Pipistrellus pipistrellus* (netting index = 0.53; n =19; 11♂, 8♀) was the most captured while *Hypsugo savii* and *Scotophilus Kuhlii* (netting index = 0.03) were the least captured among nine bat species. *Scotophilus heathii* (n = 18; 9♂, 9♀) and *P. ceylonicus* (n =15; 6♂, 9♀) were the two other captured species. Netting index for these two species was 0.50 and 0.42, respectively. Eleven *Taphozous nudiventris* and two *T. perforatus* that were hiding in their day time roosts were also captured from study area.

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**ETHNO-ORNITHOLOGICAL SURVEY, DISTRIBUTION AND  
CONSERVATION OF AVIFAUNA IN THE TERRITORY OF RIVER CHENAB,  
PUNJAB, PAKISTAN**

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Ethno-ornithology is the study was conduct to enhance the interest about the protection of the birds in the territory of the river Chenab. The data was collected from the three sub regions of the study area *i.e.* SR-1, SR-2 and SR-3. The study was carried out from the May 2009 to April 2012. The interviews were conducted to know the local Punjabi name of species of birds and meetings were conducted to enhance the importance of birds. 150 species of birds (distributed as; 56 resident, 10 summer breeder, 77 winter visitor and 7 year round visitor) were recorded during the survey; they knew 147 vernacular names of bird's species out of total. Conclusion of the research is that rich avifauna diversity is present and inhabitants have much interaction with birds and negligible Human-bird conflict is present.

**IMPACT OF HUMAN ACTIVITY ON DISTRIBUTION AND ABUNDANCE OF  
TWO MONGOOSE SPECIES IN POTHWAR PLATEAU, PAKISTAN**

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Two mongoose species native to Pakistan include small Indian mongoose (*Herpestes javanicusi*) and grey mongoose (*H. edwardsii*), are carnivore in their food habits and terrestrial, diurnal hunters. These morphologically similar species are predicted to partition the resources in time and space in the Pothwar Plateau. They exploit a variety of habitats and prey species. Here we investigated the distribution and population abundance of these two species as responding of human activity, during the 20 months period starting from November 2011 to June 2013. We developed a distribution map of the two species by extensive survey of the Pothwar Plateau and conducted 12 trapping events at 12 selected sampling sites (100 ha each) to estimate their population abundance, calculated by MNA (Minimum Number Alive) through capture recapture

method (CMR). Linear- Mixed Model was fitted by REML to analyse the influence of multiple explanatory variables (species, district, months, years, human and site) on population abundance (MNA) as response variable using R package (R i386 3.0.2) for both mongoose species under investigation. Distribution and the relative abundance of the two species interacted significantly with human activity levels. Small Indian mongoose (*Herpestes javanicus*) are more distributed near human habitation and also the population abundance of small Indian mongoose increased as human activity levels increased while gray mongoose (*Herpestes edwardsii*) shows avoidance to human and their abundance declined with the increase of human activity level.

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### BAT FAUNA DIVERSITY OF LAHORE, PAKISTAN

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Present survey was conducted to find out the microchiropteran diversity of Lahore city. During survey a total of 52 individuals belonging to 3 genera and 5 species were captured. These species include *Rhinolophus blasii* (n = 3), *Scotophilus heathii* (n = 18), *S. kuhlii* (n = 4), *Pipistrellus pipistrellus* (n = 20) and *P. ceylonicus* (n = 7). The external body, cranial and bacular measurements of selected bat specimens were taken and compared with available literature.

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### BAT FAUNA OF DISTRICT BAHAWALPUR

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A survey to find out the microchiropteran fauna of district Bahawalpur was conducted in 2011 and a total of 43 individuals belonging to 3 Families, 5 Genera and 6 species were captured from the study area. The total netting effort was 3600 m<sup>2</sup>hrs with overall mist netting index of 0.47. *Rhinopoma hardwickii*, *Taphozous nudiventris* and *T. perforatus* were captured from their daytime roosts with the help of hand net. *Pipistrellus pipistrellus* was the most recorded (netting index = 0.33) while *Scotoecus pallidus*

(netting index = 0.06) was the least recorded species among those captured through mist nets. *Taphozous nudiventris* (34.9%) and *Pipistrellus pipistrellus* (27.9%) were the most abundant bat species of the area.

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### **FOOD CONTENTS ANALYSIS OF WATERFOWL PASSING THROUGH INDUS RIVER AT TAUNSA**

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A study was conducted to investigate the food contents of migrating waterfowl in Bet Makwal Kalan near Head Taunsa Barrage, along the River Indus passage. A sum of 459 gizzard samples was collected, during their Fall migration, relating to 10 Waterfowl species *i.e.* Northern Pintail (n=56), Eurasian Wigeon (n=17), Garganey (n=07), Mallard (n=02), Northern Shoveler (n=22), Common Teal (n=129), Gadwall (n=158), Tufted Duck (n=04), Common Pochard (n=29) and Ferruginous Duck (n=34). The Waterfowl species preferred to consume plants over animals as food during winter migration, while passing at Taunsa. Food analysis showed that all ten bird species ingested seeds abundantly. Two plant species (*Najas marina* and *Potamogeton pusillus*) were consistently among the most consumed seeds in six species of ducks including Eurasian Wigeon, Garganey, Mallard, Gadwall, Tufted Duck and Common Pochard. Whereas, the seeds of *Potamogeton pectinatus* and *poligonum* spp. were found abundant in the diet of four ducks species, *i.e.*, Northern Pintail, Northern Shoveler, Common Teal, and Ferruginous Duck. In animals, molluscs and crustacean were well represented in gizzard samples. Additionally, the study highlights about the importance of vegetations in wetland ecosystem.

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### **INTENSITY OR EXTENSITY IN FORESTRY: FACTORS INFLUENCING DECISION MAKING ON THE INTENSITY OF STATE FOREST MANAGEMENT**

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State forest services act as a back bone for the management of the state forests. Unlike the private timber enterprise, for which maximum production and profit maximization is the main purpose, state forest service has to play a multidimensional role. It has to take care of the forests but on the other hand it is liable to care for the society needs and demands. In such a multifunctional liability, the "intensity" of

management appears as a helping tool with the forests service through which it gets its objectives harnessed. In order to do so, state forest service makes important decisions for management intensity under the forest policy rules for the management of the forests. Here two situations come across the forest service; either it strictly abides by the policy rules having no freedom of choice to select an appropriate intensity level. The second situation is that it has freedom of choice to make combinations of different intensity levels, but at the same time there is a need to decide for low or high management intensity levels. At this point some factors intervene which influence their decision-making. These factors play a role in their decisions for a certain level of management intensity. In this research, it is tried to find out these influencing factors which make a baseline for the decisions of the state forestry. The results came up with some prominent factors that are relevant to the decision-making by the state forest service. These are namely society, ecology, economy and policy.

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**MANAGEMENT PRACTICES IN MULTI - DESIGNATED PROTECTED AREAS: A CASE STUDY OF LAL SUHANARA NATIONAL PARK**

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The mission of protected areas has expanded from a narrow focus of biodiversity conservation to community based natural resource management for human welfare from last three decades or so. Consequently, worldwide there is a shift in protected areas management which allows involvement of local community in resource management for example in case of Man and Biosphere Reserve (MAB) Program. However, in case of Pakistan, protected area management practices still limited to protection of the boundaries allowing minimal community interaction. Present study is designed to identify the factors which limit community based natural resource management in Pakistan. Lal Suhanra National Park (LSNP) has been selected as case study due to its unique combination of desert, natural lake and forest area. LSNP is also an area under different designations of international and national systems *i.e.* National Park of Pakistan declared by Punjab Wildlife Ordinance 1972, IUCN Category V and Biosphere Reserve (MAB program). Present study mainly deals with the analysis of current management practices in LSNP with reference to its three designations, based on information that is gathered through primary (site survey and interviews) and secondary data (legislations and guidelines for management of protect area). Our study shows that LSNP is facing threat of degradation in all of its three habitat areas and also local community conflict mainly due to management guided by weak and out dated legislation, negligence of its designated authorities, institutional rift among government departments and absence of

local stakeholders involvement. Clear chain of command, coordination among departments and stakeholders, community based management and arrangement for extensive research activities are required to cope up current management deficiencies.

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### **THE PHARMACOKINETICS OF NSAID'S BIOASSAY IN COMMON QUAIL**

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The objective of the present study was to bioassay the pharmacotherapeutics and pharmacokinetics of five different NSAIDs (meloxicam, mefenamic acid, ibuprofen, diclofenac potassium and paracetamol), in common quail (*Coturnix coturnix*). A total of 120 birds were used for *in-vivo* investigation. The study was estranged into two trials over the winter and summer seasons, each comprised of both ten days and twenty days long experiments. Each study group encompasses control (n=10) and experimented birds (n=50). A sum of 5 groups' representing five different "NSAIDs' trial-groups" was treated with therapeutic doses at the stretch of ten days and twenty days in each season. A therapeutic dose of each NSAID was given at the rate of: meloxicam 1.8 mg/kg, mefenamic acid 2mg/kg, Ibuprofen 2mg/kg, diclofenac potassium 2mg/kg and paracetamol 2mg/kg to each bird per dose x 3/day. The clinical signs including depression, somnolence and increased body temperature ( $p<0.01$ ) were exhibited in all (100%) treated birds. Nevertheless, only in summer trials, a substantial amount of swelling ( $p<0.01$ ) on feet was observed in all treated birds. Whereas, some mortality (2.5%) was seen in case of mefenamic acid, meloxicam and diclofenac potassium treated groups. A decrease in body weight was observed only in diclofenac potassium trials. However, there were no signs of avian visceral gout observed in all cases. Some sort of mild swelling (29%) was experienced in kidney and liver of some treated birds. There was no apparent difference in histological analysis of heart and skeletal muscles in all treated birds with that of the control group. However, in case of diclofenac, a substantial amount of kidney and liver impairment was observed as compared to other treated groups that showed mild changes.

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**SECTION - VI****POSTER SESSION****PUBLIC CONCERN ON COPPER AND IRON TOXICITY IN RADISH  
(*RAPHANUS SATIVUS*) AND SPINACH (*SPINACIA OLERACEA*) IRRIGATED  
WITH WASTE WATER**

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Illegal cultivation of vegetable is a common practice in soil irrigated with municipal wastewater. An experiment was conducted in District Sargodha to assess some heavy metals concentrations in vegetables irrigated with wastewater. Different sites of District Sargodha were selected to estimate the level of iron and copper in soil and vegetables irrigated with waste water. Analysis of soil, water and vegetables revealed that concentrations of iron and copper in radish and spinach irrigated with waste water were observed high than FAO recommended levels. By the use of waste water, large concentration of iron and copper accumulated in soil and edible part of vegetables. Excess concentration of heavy metals in soil, water and vegetables disturbs the natural ecosystem. It has been observed that large quantity of untreated municipal wastewater becomes the source of metal pollution.

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**COMPARATIVE PROXIMATE BODY COMPOSITION OF WILD AND  
FARMED FRESHWATER FISH, *CIRRHINUS MRIGALA***TANVEER AHMED<sup>1,\*</sup>, SYED MUHAMMAD AUN NAQVI<sup>2</sup>, SAJID ABDULLAH<sup>1</sup>  
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The proximate composition of flesh (muscle), head, scales, fins and skin of wild and farmed *Cirrhinus mrigala* were analyzed in the present study. The purpose of present study was to analyze nutritional value of wild and farmed *C. mrigala* and their comparison with each other. *C. mrigala* is a major carp and commonly consumed throughout Pakistan due to its high production and its taste. Seven specimens of *C. mrigala*, of three different weight categories ( $\leq 500$  g, 501-1000 g and 1001-1500 g) were collected from both wild and farmed habitat. High moisture contents were noted in the wild and farmed *C. mrigala* having weight  $\leq 500$  g  $71.57 \pm \%$  and  $67.43 \pm \%$  respectively. It is observed that moisture contents decreased as the weight of specimen increased.



Farmed or cultured *C. mrigala* showed significantly ( $p < 0.05$ ) high protein and lipid contents in all body parts as compared to wild species. Ash percentage was observed significantly ( $p < 0.05$ ) high in those body parts that have large bone matrix including head, scales and fins of both wild and farmed species. It was found that although scales and fins of wild and farmed *C. mrigala* were not consumed directly by human beings, they have considerable amount of nutrients. Farmed *C. mrigala* was found better on the basis of nutritional and commercial value than that of wild *C. mrigala*. This article will help the fish consumer to know the nutritional value of wild and farmed *C. mrigala*. This information is beneficial for nutritionists that concerned readily available source of energy and for food scientists who are seeking high quality proteins with acceptable flavor, texture, color and odor along with safety to consumers. It also facilitates the selection of most appropriate species having higher protein content and optimum size and condition for human consumption.

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**IDENTIFICATION AND BIOLOGICAL SCREENING OF WILD MUSHROOMS  
FROM AZAD JAMMU AND KASHMIR AGAINST MUTATED HUMAN  
PATHOGENIC BACTERIA**

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Mushrooms are rich in carbohydrates and proteins. They have several biological activities like anti-proliferative, antiviral, antifungal and antibacterial effects. In the current research fourteen different mushrooms were collected from Neelum Valley, Azad Jammu and Kashmir, Pakistan. Mushrooms were identified and used to evaluate their antimicrobial and antioxidant activities. Acetone, ethanol, propanol and DMSO solvents were used for extract preparation. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Serratia marcescens* were used as tested microbes. Determination of free radical scavenging activity, total phenol and flavonoid contents by spectrophotometry were also carried out. Antioxidant activity was also identified by DPPH as spray reagent after separating the compounds by thin layer chromatography (TLC). Propanolic extracts of almost all mushrooms showed significant inhibition of non mutant type of *P. aeruginosa*, mutant two (M2) types of *S. marcescens*, mutant four (M4) type *S. epidermidis*. Whereas interestingly it was observed that almost all extracts of mushrooms have significant inhibition effect on mutant four (M4) types of *S. aureus*. It was concluded that mushrooms had a great potential for the production of useful bioactive metabolites and that they may be prolific resource for drugs.

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### **A REVIEW OF FOOD AND FEEDING HABITS FOR FRESH WATER FISHES FROM PAKISTAN**

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Riverine fish growth mainly depends upon the planktonic richness and other particulate food matter. Accordingly fish gut analysis reflects the availability of nutrients and hence the growth profiles of the animals. In this review the gut content analyses of above fifty fish species representing different fresh water habitats from Pakistan is reported. The information are relevant for aquaculturing as well as rehabilitating the riverine habitats. While the fish species for which the relevant data are not available have been highlighted for future studies.

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### **FUNGAL AND BACTERIAL INHIBITION THROUGH DEODAR (*CEDRUS DEODARA*) AND TIMUR (*ZANTHOXYLUM ARMATUM*) EXTRACTS AND THEIR PHYTOCHEMICAL SCREENING**

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*Zanthoxylum armatum* and *Cedrus deodara* are important medicinal plants of Pakistan and have both antimicrobial and antioxidant activities against pathogenic bacteria as well as fungi but these plants are badly neglected in Pakistan. The aim of study was to evaluate the antimicrobial, antioxidant activities and phytochemical screening of these medicinal plants. Fungal and bacterial pathogens such as *Aspergillus*, two *Rhizopus* spp, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Serratia marcescens* were used as tested microbes. Through agar disc and well diffusion methods it was observed that 100% concentration of all extracts of *Z. armatum* and *C. deodara* exhibited both antibacterial and antifungal activities. Saponins, tannins and glycosides were observed in all extracts of *Z. armatum* whereas proteins, amino acids, phalobatanins, and carbohydrates were not found. Similarly saponins, tannins, carbohydrates and glycosides were observed in all extracts of *C. deodara* whereas proteins, amino acids, phalobatanins were absent. Thin layer chromatography profiling of all extracts also gave an idea about the presence of various phytochemicals. Spray of

DPPH on thin layer chromatographic plates indicated the presence of antioxidants. Significant inhibition of *Serratia marcescens* and *Staphylococcus aureus* was also observed through TLC-Bioautography. These results suggest that both *Z. armatum* and *C. deodara* are potential source of plants for controlling pathogenic microbes.

### **PREVALENCE OF FASCIOSIS IN BUFFALOES OF BAHAWALPUR, PUNJAB, PAKISTAN**

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Fasciolosis is a trematode borne parasitic disease that infects liver of large ruminants widely prevalent throughout the world. During the present study fecal samples from buffaloes were collected on random basis from the all tehsils of Bahawalpur district from February 2012 to October 2012. Of total 1800 fecal samples, 284 (15.8%) were found to be positive. Highest prevalence was recorded in Yazman (21.7%) followed by Bahawalpur (16.7%), Khairpur (15.6%), Hasilpur (14.4%) and the lowest was recorded in Ahmedpur (10.6%). Statically chi-square showed non significant ( $p>0.05$ ) difference between all areas. Monthly overall highest prevalence was recorded in September (31%), while the lowest was found in the month of May (3.5%). Statistically a significant ( $p<0.05$ ) difference was recorded in all months. Overall highest seasonal wise prevalence was found in autumn (28.3%) followed by winter (21%), summer (12%) and lowest in spring (8.3%). In age wise prevalence the adult buffaloes were highly (19.9%) infected than young ones (5.3%). Statistically a significant difference ( $p<0.05$ ) was found between all seasons and age groups. Gender wise the prevalence was slightly higher but statistically non significant ( $p>0.05$ ) in females (15.9%) than males (15.1%).

### **ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITIES OF MORUS NIGRA EXTRACTS**

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*Morus nigra* possesses several medicinal values, is used as therapeutic agent, non toxic and it belongs to the family *Moraceae*. The aim of the present study was to

evaluate the antimicrobial and antioxidant activities of fruits extracts of *M. nigra* prepared in methanol, ethanol, acetone, chloroform and diethyl ether extracts. Phytochemical screening and DPPH free radical scavenging activity were also investigated. Total phenolic and total flavonoid content was estimated by folin-ciocalteau and aluminium chloride methods, respectively. Significant growth inhibition of all tested bacteria was recorded by *M. nigra* extracts as compared to fungal microbes. The phytochemical analysis showed that glycosides, tannins, saponins, and alkaloids were present in all fruit extracts of *M. nigra* whereas amino acids were absent. It was observed that phenolic and flavonoid contents were higher in amount in ethanolic and acetone extracts as compared to others. Thin layer chromatography technique was employed to enumerate the antioxidants and phytochemical constituents in most active solvent extracts by using various solvent systems. The compounds contained in these extracts over silica gel were also evaluated through bioautography.

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#### **ISOLATION OF ACETONE YIELDING BACTERIA AND THEIR CULTIVATION EMPLOYING LOW COST SUBSTRATES**

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Acetone is a widely demanded general purpose organic solvent as well as active ingredient of various cosmetic and other industrial products. Forty bacteria were isolated from 10 soil samples representing different vertical locations of agricultural and garden habitats. Ten of the isolates gave positive test with sodium nitroprusiate and ammonium hydroxide. Of these ten isolates different bacteria grew differentially in water melon pulp, potato peels, sugar cane bagasse, molasses and pure cellulose based media. The local isolates represent acetone producing bacteria capable of yielding the organic solvent from the agri-industrial wastes. The bacterial isolates have the potential of developing economically feasible process for acetone production from the waste bioresources.

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#### **ANTI-PROLIFERATIVE ACTIVITY OF INDIGENOUS ACTINOMYCETES ISOLATED FROM DIFFERENT HABITATS OF PAKISTAN: PRESCREENING AND RIBOTYPING.**

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Actinomycetes are a group of Gram positive bacteria with high G+C Content. This group of bacteria is capable of producing different types of novel secondary

metabolites. Many of these metabolites possess different biological activities and have the potential to be developed as therapeutic agents. The aim of the present study was to screen the indigenous actinomycetes for cytotoxic and anti-proliferative compounds. In our screening, several water and soil samples were collected from various habitats of Pakistan. More than 500 isolates were isolated, 120 of which were selected for initial identification and cytotoxic profiling. The strains were characterized on the basis of their Morphological, Biochemical and Physiological behavior. In a biological screening the crude extracts obtained from the culture broth of selected strains were analyzed for their cytotoxic activity through Brine shrimp microwell cytotoxicity assay. The isolates with high larvicidal activities were then tested for anti-tumor or anti-proliferative activity against various proliferative cell lines (Hela, MD-BK, Vero cell lines) through methyl thiazolyl tetrazolium (MTT) bioassay method. 20 isolates were selected with high inhibition rate against proliferative cell lines. These selected isolates were then genetically characterized through 16S-rRNA sequencing and was found that majority of these strains were belonging to a well known family of actinomycetes named as *Streptomyces*.

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#### **BIOLOGICAL STUDIES AND PHYTOCHEMICAL SCREENING OF *ATROPA ACCUMINATA* AND *ATROPA BELLODONA* EXTRACTS**

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*Atropa accuminata* and *Atropa bellodona* were being used as medicinal plants against various infections. The antioxidant constituents were analyzed using thin layer chromatography followed by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) technique. Antibacterial activity against nine bacterial and three fungal pathogens was analyzed through well diffusion methods. Sensitivity test of antibiotics was also recorded by agar disc diffusion method. Total phenolic and total flavonoid contents were also estimated by folin-ciocalteau and aluminium chloride methods. TLC (thin layer chromatography) spot screening of both medicinal plants indicated the presence of antimicrobial agents. *A. belladonna* and *A. accuminata* extracts showed low antifungal activity against *Aspergillus* whereas other two fungal pathogens are susceptible. Phenolic contents were higher in ethanolic and DMSO extracts of *A. bellodona* roots as compared to leaf extracts whereas flavonoids are more in DMSO extracts of *A. bellodona* leaf. Such findings would be useful in promoting research aiming at the development of new antimicrobial agents and antioxidants.

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**ISOLATION OF ACID TOLERANT LAB ANTAGONISTIC TO COLIFORM AND STAPHYLOCOCCUS AUREUS**

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Eight lactic acid bacteria (LAB) procured from dairy products associated habitats were found acid tolerant. The select LAB isolates were processed through cross streaking for screening their antagonism against coliform and *Streptococcus aureus* bacteria. The LAB which depicted antagonistic activities against the tested bacteria are good potential candidates for fermenting dairy products with promising health benefiting effects by controlling the disease causing bacterial contaminations during as well as the post fermentation processes. Application of such LAB is likely to enhance shelf life of the fermented dairy products by reducing the chances of bacterial contaminations in addition to various known and presumptive health promoting effects of the probiotics.

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**PEOPLE'S PERCEPTION ABOUT POOR QUALITY OF DRINKING WATER AND ITS IMPACT ON HUMAN HEALTH IN RURAL AREAS**MUHAMMAD NADEEM<sup>1</sup>, SHAZIA YASMIN<sup>1</sup>, MUNWAR IQBAL<sup>2</sup>, MUHAMMAD NADEEM ABBAS<sup>3</sup> AND TAHIR IQBAL<sup>3</sup><sup>1</sup>*Institute of Agri. Extension and Rural Development, University of Agriculture Faisalabad*<sup>2</sup>*Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad*<sup>3</sup>*Department of Zoology, University of Gujrat, Gujrat, Pakistan*Email: [randhawa136@yahoo.com](mailto:randhawa136@yahoo.com)

The present survey study was conducted to assess the perception of common men about the impact of poor quality drinking water on human health in rural areas of Tehsil Samundri, Faisalabad (31.2500° N, 73.0500° E). Overall 110 respondents were interviewed and information was collected through questionnaires. The most respondents were used hand pumps (78.2%) or electric pump's (63.6%) water for drinking purpose. Little less than one-fourth (22.7%) of the respondents were satisfied 'to a great extent' and 31.8% of them were satisfied 'to some extent' with the quality of water supplied from the main source. About one-fourth (24.5%) of the respondents reported that they were never satisfied with the quality of water due bad quality/bad smell. Respondents reported that their family members suffered waterborne diseases *i.e.* Diarrhea (37.3%), Cholera (26.7%), Viral hepatitis (40.0%), and Gas trouble/ gastroenteritis (53.6%). So it is suggested that water quality should be improved by the Government, and non-government institutes to raise the living standard and to save from diseases in the study area.

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***PROGIRAFFA* (ARTIODACTYLA, RUMINANTIA, GIRAFFIDAE) FROM CHINJI FORMATION OF LOWER SIWALIKS, NORTHERN PAKISTAN**

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Attached frontal appendages of *Progiraffa* have been discovered from the Lower Siwaliks of Pakistan. The material is the first so far Neogene land mammal site found in Pakistan. Some mandible and maxillary fragments can be assigned to *Progiraffa* on the basis of their size were also collected from the Chinji Formation (14.2-11.2 million years ago) of the Pakistani Lower Siwaliks. The fossils are sufficiently interesting to warrant description one of the earliest members of the family Giraffidae. The fossils range in age between 16 and 11.2 million years old and together with species of tragulids provide evidence for the existence of diverse Earliest Miocene and Middle Miocene ruminant faunas in southern Asia.

**CITRIC ACID AND PHYTASE SYNERGISTICALLY ENHANCE THE NUTRIENT DIGESTIBILITY IN *LABEO ROHITA* FINGERLINGS**

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Present research work was planned to determine any possible synergistic effect of citric acid and phytase to improve nutrient digestibility performance in *Labeo rohita* juveniles. The experiment was conducted in Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of Agriculture Faisalabad. The experimental diets were formulated by mixing soybean meal, fish meal, rice polish, wheat flour, fish oil, minerals, vitamin premix, chromic oxide and ascorbic acid. A uniform mixture of all these ingredients was obtained by blending them in mixture machine. The uniform mixture was acidified with the addition of three different levels of citric acid (0, 1.5% and 3%). The acidified diet was further supplemented with three graded levels of PHY (0 FTU kg<sup>-1</sup>, 750 FTU kg<sup>-1</sup> and 1000 FTU kg<sup>-1</sup>). During feeding trial, triplicate tanks were used having 10 fish. Fish were fed twice a day. The diets were given 2% of the live wet weight of fish. After two hours of feeding session tanks were washed out to get rid of uneaten feed

particles. Fecal collection was done after two to three hours of feeding session by opening the valve fitted at the base of tanks. After collection, fresh feces were placed in oven (at 60°C) for drying. 5 to 6 g feces of each diet were collected for nutrient analysis. The findings of the recent research are here

- The main effect data of citric acid and phytase addition indicated improved dry matter, crude protein, crude fat and gross energy digestibility in rohu juveniles fed different diets.
- Interaction data showed no clear change ( $p > 0.05$ ) in dry matter and crude protein digestibility while significant synergistic effect was observed for crude fat and gross energy.
- Likewise, both the supplements (citric acid and phytase) also improved the mineral digestibility independently as well as in combination.

In conclusion, addition of citric acid and phytase in soybean meal based diets improved the nutrient and mineral digestibility performance.

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#### HISTOPATHOLOGICAL CHANGES INDUCED BY IMIDACLOPRID INSECTICIDE IN TESTIS OF RABBITS

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This study was aimed to know the toxic effects of Neonicotinoid (Imidacloprid) insecticide on the male gonad of rabbit. In this context, a total of 20 rabbits, *Oryctolagus cuniculus*, were used as one test group whereas second group labeled as control. One tenth of LD<sub>50</sub> imidacloprid 200 SL was orally administrated in test group animals for 15 days. At the termination of experiment all animals dissected and testes were removed in order to evaluate the testicular weight subsequently pieces of removed testes were fixed for histological examination. The testicular weight in the rabbits exposed to imidacloprid for 15 days was reduced highly significantly ( $P < 0.01$ )  $3.62 \pm 0.10$  grams as compared to the control group  $4.06 \pm 0.06$ . Body weight was significantly ( $P < 0.05$ ) decreased in the treated animals. Histopathological observations revealed that, the interstitial space as compared to control became widened and the number of leydig's cells was also decreased. Tumor formation was noted in the interstitial space which was in initial stage. It is concluded that exposure of imidacloprid insecticide exerted disruption in testes and showed adverse effect on the testicular tissues in rabbits which probably can cause reproductive disorders.

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**MOLECULAR CHARACTERIZATION OF BACTERIAL STRAINS ISOLATED  
FROM CARTAP HYDROCHLORIDE AND IMIDACLOPRID APPLIED  
AGRICULTURAL SOIL**

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Bacterial strains from Cartap hydrochloride and Imidacloprid applied soil from District Gujrat, Pakistan were isolated and cultured. All the strains were found to be gram positive and cocci in shape. Plasmid profile analysis showed plasmids with almost 23.13kb, 1.1kb and 0.75kb sizes. Two sets of primers were optimized for 16S rRNA gene amplification through Polymerase Chain Reaction (PCR) sequence for the molecular identification of bacterial strains. The product sizes obtained were 996bp and 992bp, which may be used for molecular identification on the basis of 16S rRNA gene sequence. These bacterial strains may be good bioremediation tool for organophosphate pesticides removal from environment.

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**IN VITRO INHIBITORY ACTIVITY OF PLANT EXTRACTS AGAINST  
BIOFILMS FROM ORAL SAMPLES, FOOD INDUSTRY AND CLINICAL  
SAMPLES (URINE AND WOUNDS)**

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Biofilms are universal occurring in large number of environment ranging from aquatic, food industries to medical devices thus posing great risk to human health. Microorganisms growing in a biofilm are highly resistant to antimicrobial agents and they cause several human diseases. Hence the objective of this study is to assess the biofilm forming capacity of bacteria. For this purpose, oral sample from 30 subjects, clinical samples from 30 subjects and 30 industrial samples were collected. 150 bacterial strains were isolated out of which 75 were purified on the basis of morphological variations on nutrient agar plates. 30 strains showing high antibiotic resistance profile were selected and characterized further on basis of biochemical tests. Biofilm characterization was done for 9 strains (F<sub>22</sub>, B<sub>4</sub>, SpG<sub>6</sub>, U<sub>3</sub>H, W<sub>21</sub>cb, U<sub>15</sub>A, Sh<sub>1</sub>(1), F<sub>2</sub>(2) and S<sub>7</sub>(2) following three methods *i.e.* Congo red, liquid interface coverslip method and test tube method. Selected strains were tested for their susceptibility to the aqueous and methanolic extracts of five (Garlic, Banana, Piper mint, Clove and Green tea) Plants in both planktonic and biofilm mode. The effect of plants in planktonic mode was tested by agar well diffusion method. Results showed both gram positive and gram negative strains. Physiological characterization showed 37°C as optimum temperature for all strains, however all strains

showed best growth pH 7 except B<sub>4</sub> which showed best growth at 6. Biofilm formation by Congo red method resulted in black colonies for 6 strains which were further confirmed by cover slip after 24hr and test tube method having maximum biofilm formation after 72 hours. Aqueous extract of garlic showed highest antibacterial activity for all strains. The methanolic extracts of plants showed more antibacterial affect than aqueous extracts except garlic.

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### **ECOLOGICAL ASSESSMENT OF NARMADA RIVER WITH SPECIAL REFERENCE TO DIVERSITY OF MOLLUSCAN SPECIES**

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The molluscan species are helpful in purification of water in their capacity to act as scavengers. Narmada river is one of the most important river of India, which covers 98,796 sq. km of total water shed area. Narmada is considered to be life line and west flowing river of state Madhya Pradesh. Limnological study was carried out for a period of eight months from Aug 2009- March 2010 in selected stations of Narmada river. In present study various species of molluscans belonging to class gastropoda and pelecypoda were recorded. Among gastropods the *Vivipara benglensis* was dominant followed by *Bellamya benglensis* in both stations. Among pelecypods *Perreysia caerulea* was dominant throughout the study period. Highest Shannon and Weiver index (H) was observed in station II and lowest in station I. The minimum H value was observed in December 2009 and maximum in March 2009. The result of the present study emphasizes the importance of conserving the world's freshwater molluscan population, which are declining at an alarming rate through habitat destruction and pollution.

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### **ISOLATION OF ARSENIC RESISTANT BACTERIA FROM SOIL AND WATER SAMPLES OF LAHORE AND KASUR**

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Arsenic is an extremely toxic metalloid, when present in high concentration severely threatens the biota and human health. Microbes possess the potential of arsenic accumulation within their cell and can also transform toxic arsenite As III to less toxic arsenate As V. The present study was focused at isolation and optimization of arsenic resistant bacteria of soil and water samples taken from Lahore and Kasur arsenic

contaminated areas. Eleven bacterial isolates were isolated on sodium arsenite containing media FA-I. Three isolates FA 1, F A4 and FA 9 from Kasur area were selected on the basis of arsenic tolerance (100 mM). At 100mM arsenic concentration FAs1 had  $7.48 \times 10^9$ , FAs 4 had  $1.57 \times 10^9$  and FAs 9 had  $2.23 \times 10^9$  CFU/ml. Further these isolates were optimized under different physical conditions to observe their maximum arsenic tolerance and bioaccumulation potential. At 37°C (optimum) FAs1, FAs4 and FAs 9 had increased growth *i.e.*,  $2.48 \times 10^8$ ,  $5.33 \times 10^8$  and  $3.16 \times 10^8$  C.F.U./ml, respectively. Whereas optimum pH was 7 for isolate FAs1, FAs4 and FAs9 that showed  $4.38 \times 10^8$ ,  $4.40 \times 10^8$  and  $4.43 \times 10^8$  C.F.U./ml of growth. Maximum arsenic concentration tolerated under optimum conditions for the bacterial strains F As 1, FAs 4 and FAs 9 was 80mM. These isolates had less redox ability to oxidize arsenite As III to arsenate As V. F As 1, F As 4 and F As 9 showed arsenic accumulation to extent of 39.16, 148 and 125 µg/L on the 4th, 3<sup>rd</sup> and 4th day of incubation, respectively. Isolate F As 1 and F As 2 were gram negative and endospore forming rods whereas F As9 was gram negative cocobacilli. Based on the current study these novel isolates possess a great potential in biotechnology field. Bioremediation of arsenic contaminated soil and water by employing arsenic resistant as well as accumulating bacteria is a compatible mode of environment clean up and is cost effective.

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#### **EFFECT OF ANCHOR POINTS ON WEB STRUCTURE OF *NEOSCONA THEISI***

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The size and shape of webs is largely determined by the choice of anchor points and length of anchor threads. Experiments were designed to study the effects of anchor points on the web size, web shape, web asymmetry and web geometry (mesh height, capture thread length, web capture area and number of radii) of *Neosconatheisi*. For experiment 30 plots (3x3 m) were maintained into three treatments *i.e.* control (400 tillers/ m<sup>2</sup>), halftinning (200 anchors/ m<sup>2</sup>) and three fourth thinning (100 anchors/ m<sup>2</sup>) of plants. In laboratory, spiders were released in wooden cages having the areas 60x60 cm, 60x30 cm, 30x60 cm and 60x15 cm. Results showed that web parameters *i.e.* mesh height, web size, web area, capture area and capture thread length were significantly larger when exposed to very thin vegetation as compared to control vegetation. However, capture thread length and number of radii were more in this vegetation as compared to control. The laboratory studies indicated that the available space determined the placement and design of webs. The web size was reduced in the small size frame as compared to large frames.

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**EFFECT OF NEONICOTINOID INSECTICIDES ON THE SURVIVAL AND DETOXIFYING ENZYMES OF LYCOSID SPIDER (*PARDOSA OAKELYI*)**

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Toxic effect of neonicotinoid insecticides *i.e.* imidacloprid and acetamiprid was assessed on detoxifying abilities of ground spider *Pardosa oakelyi* in the laboratory. Bioassay showed that *Pardosa oakelyi* varied in the susceptibility of both insecticides. At the field rate, Acetamiprid was 2.5 times more toxic than imidacloprid. Quantity of detoxifying enzymes Acetylcholinesterase (AChE), Carboxylesterase (CarE), Glutathione S-transferase (GST) and Cytochrome Oxidase P450 varied with the concentration of both insecticides. Carboxylesterase and Cytochrome Oxidase P450 were negatively correlated with the concentration of insecticides while AChE and GST did not show any correlation with both insecticides. In spiders treated with Acetamiprid, AChE, CarE, GST and oxidase P450 values varied significantly from the control groups. While in imidacloprid treated spiders, AChE values did not differ from the control spiders. However CarE, GST and Oxidase P450 values of control and treated spiders were significantly different from each other. Results of the present study showed that Acetamiprid is highly toxic and disrupts the natural detoxifying system in the studied spiders.

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**DETECTION AND PHYLOGENY OF *WOLBACHIA PIPIENTIS* IN *BACTROCERA ZONATA* FROM THREE DISTRICTS OF KHYBER PAKHTUNKHWA, PAKISTAN.**NAQIBULLAH KHAN\*, BILAL RASOOL, KASHIF ZAHOOR, SHABAB NASIR  
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*Wolbachia* are a common and widespread group of endosymbiotic bacteria which is capable of manipulating reproduction of arthropod and nematode hosts. They are maternally inherited and settle up to 76 % of all insect species. This bacterium causes a variety of reproductive abnormalities including feminization, male killing, parthenogenesis and most commonly cytoplasmic incompatibility (CI). Recently, it has been testified as a possible means for insect pest population control. Fruit flies are among the agricultural pests that have great economic importance. A major research aim of the current study is the exploration of *Wolbachia* strains from Khyber Pakhtunkhwa fruit fly populations (*Bactrocera zonata*) leading towards its phylogenetic narrations through molecular markers. Collection of fruit fly adults was performed from three districts

(Bannu, Kohat and Peshawar) of Khyber Pakhtunkhwa. Different life stages were collected from infested fruits, reared in the Lab and then stored in 70% ethanol at -20°C prior to DNA extractions and Some of the populations were found to be infected with *Wolbachia* through PCR using wsp (81F and 691R) primers. Sequences were engendered which will further be analyzed for phylogenetic studies. The current research work would help reduce the use of hazardous pesticides in Fruits and vegetables and also promote the use of *Wolbachia* as a biocontrol agent.

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### BIOLOGICAL CONTROL OF MOSQUITO LARVAE USING EDIBLE FISHES

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Various methods aimed at eliminating mosquito have been tried. However there are certain environmental issues related with chemical control of mosquitoes using insecticides. Biological control offers safe way of hitting the weakest life cycle link. Present study is focused on using edible fishes as predator of mosquito larvae. Experimental results reveal that edible fishes have tremendous potential to be used as larvivorous predator. It was further found that fish under study prefer live larvae when compared with formula feed, and temperature fluctuations have correlation with larval consumption.

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### STK11 MUTATION IN PEUTZ-JEGHERS SYNDROME; AN AUTOSOMAL DOMINANT CONDITION

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Peutz-Jeghers syndrome (PJS) is an autosomal dominant condition with multiple polyps throughout the gastrointestinal tract, pigmented lesions of the vermillion border of the lips, in almost all patients. PJS patients have an increased risk of developing intestinal malignancies as well as other types of cancer. The cause for PJS is germ line mutation of a Serine/threonine kinase 11 (STK11; 50-kDa); a Tumor suppressor gene located at 19p13.3. Until recently, over 230 different STK11 mutations causing the PJS have been described in the Human Gene Mutation Database (HGMD). Most of them are small mutations including: 72 point missense/nonsense mutations, 26 mutations in splicing sites as well as 102 small point mutations. The remaining 31 mutations including 25 large deletions, 3 large insertions and 3 combined mutations. The Disease pathway map of PJS is analyzed by using KEGG Disease Database, which indicates association

of genes and molecules to diseases. STK11/NY-REN-19/LKB1 (433 a.a) controls the activity of AMP-activated protein kinase (AMPK) family members, thereby playing a role in various processes such as cell metabolism, cell polarity, apoptosis. In order to get insights into this malfunction protein, we studied the predicted protein-protein interaction in *Homo sapiens* using STRING database. The immediate interacting proteins of STK11 are; STE20-related kinase adaptor alpha (STRADA; 431 a.a), Calcium binding protein 39 (CAB39/M025; 341 a.a), Cell division cycle 37 (Cdc37; 378 a.a), STK11-interacting protein (STK1IIP; 1099 a.a), STE20-related kinase adaptor beta (STRADB; 418 a.a). The STRAD ( $\alpha$  or  $\beta$ ) activates STK11 by forming a heterotrimeric complex with STK11 and the scaffolding protein M025. Cdc37 form a complex with Hsp90. This Hsp90-cdc37 complex controls both the stability and activity of the STK11 kinase. STK1IIP is found in a ternary complex composed of STK11, STK1IIP and SMAD4, regulate STK11 function by controlling its subcellular localization. In conclusion, mutations in the STK11 impair the enzyme's tumor suppressor function; allowing cells to divide too often, lead to the formation of polyps and cancerous tumors.

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#### **PRODUCTION OF BIOCHARS WITH GREATER SURFACE ACTIVITY THROUGH ALKALINE PRE-TREATMENT OF WOODY FEEDSTOCKS**

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Surface activated biochars can act as useful filtering materials as well carbon sinks. Biochars in this study were produced by low temperature pyrolysis of fibrous debarking waste from pine and eucalyptus (feed stocks) that had, or had not, been pre-treated with either diluted (L) or in undiluted (S) alkaline float tannery waste (L-PI, S-PI, L-EU, S-EU). Biochars produced from untreated feed stock were used as control treatments (Ctr-PI and Ctr-EU). The chemical and structural properties of the different samples were characterised by FT-IR, <sup>13</sup>C-MAS NMR, XPS, SEM microphotographs, and gas adsorption measurements (BET). Elemental composition, fixed C, yield, surface charge, effective cation exchange capacity (ECEC), and NH<sub>4</sub><sup>+</sup> sorption and desorption properties were also studied. Biochars made from L-EU and S-EU contained less fixed carbon (39 and 37 %, respectively) than the respective control (40 %) but showed greater

change in chemical characteristics than those made from L-PI, S-PI, which showed minimal decrease in C content compared to their controls. Differences were mainly attributed to different types of lignin (S-type / G-type) in pine and eucalyptus. The specific surface area of the biochars decreased with the alkaline treatments (from  $> 135 \text{ m}^2 \text{ g}^{-1}$  to  $< 9 \text{ m}^2 \text{ g}^{-1}$ ), although surface ECEC increased due to increased acidic surface groups. In subsequent filtration experiments treated biochars adsorbed greater  $\text{NH}_4^+$  from a 40 mg N/L waste stream (*e.g.*, 61 % retention in Ctr-EU and 83% in S-EU). Desorption of the absorbed  $\text{NH}_4^+$  was low, especially in treated biochars (0.1-2 % out of total retained) relative to untreated biochars (14-27%), suggesting the contribution of other mechanisms of  $\text{NH}_4^+$  retention, in addition to electrostatic interactions. In this preliminary experiment, pretreatment of eucalyptus fibrous bark with alkaline tannery float prior to pyrolysis was found to promote increases in surface charge and  $\text{NH}_4^+$  absorption by the biochar. The physical and chemical changes that take place in the feed stock and during the pyrolysis of the feedstock deserve further attention.

#### **IL-2 MEDIATED T-CELL ACTIVATION DURING AUTOIMMUNE DISEASE "RHEUMATOID ARTHRITIS" AND ROLE OF CTLA4**

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A polygenic disease "Rheumatoid Arthritis" is an auto immune thyroid disease that involves both environmental as well as genetic factors. Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is a diseased gene in this disease pathway having chromosomal position 2q33, AA sequence of 174 aa and nucleotide sequence of 525 nt. This in turn leads to the continuous auto immune response because of IL-2 expression. KEGG Database was used to find out mutated genes. The functional interaction partner of CTLA4 protein was studied using STRING database. The activation of T-Cells by Antigen-MHC-II complex carried on Antigen Presenting Cells is a complex process which involves the phosphorylation of the PTKs (Protein Tyrosine Kinases) belonging to the SYK ZAP70 (Zeta-Chain-Associated Protein Kinase) family. Initiation of T-cell activation is mediated by phosphorylation of the IT AMs (Immunoreceptor Tyrosine-based Activation Motifs) on the TCR-CD3 complex by Lck (attached to CD4 or CD8), and Fyn kinases. The phosphorylated ITAMs then activates the ZAP70 and SYK through phosphorylation, which magnify signals from the TCR by adaptor proteins activation: LAT (Linker Activator for T-Cells), SLP76 (SH2 Domain-Containing Leukocyte Protein-76), GADS (Growth Factor Receptor-Bound Protein-2-Related Adaptor Protein-2) and enzymatic effectors such as PLC- $\gamma$  (Phospholipase-C- $\gamma$ ) to trigger Immune Response. Signaling through CD28 promotes cytokine IL-2 mRNA synthesis leading to translation of IL-2 protein causes onset of immune response leading towards cell

proliferation, T -cell survival and T -helper- cell differentiation. On the other hand, CTLA4 Signaling inhibits IL-2 mRNA production and inhibits cell cycle progression. CTLA4 interacts with the ITAMs and essentially disrupts the biochemical signals that lead to activation of the T -Cell. ZAP70, SYK and Fyn are also potential targets of direct inhibitory interaction of CTLA4. The SNP (single nucleotide polymorphism) mutation in exon1 of CTLA4 disrupts its function leading to a continuous immune response and high level of IL-2 which is a key symptom of the auto immune disease in rheumatoid arthritis.

### **GLUCOKINASE RELATED PERMANENT NEONATAL DIABETES MELLITUS**

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Permanent neonatal diabetes mellitus (PNDM) is a monogenic form of diabetes characterized by high glucose level (hyperglycemia) in the first 6 months of life resulting from a shortage of the hormone insulin. Affected infants have hyperglycemia, dehydration and unable to gain weight and may suffer from neurological problems. PNDM can result from complete deficiency of glucokinase activity. PNDM is caused by autosomal recessive rare inactivating miss-sense mutations in glucokinase (GCK). GCK acts as a glucose sensor and stimulates the release of insulin from pancreatic  $\beta$ -cells. The GCK gene is located at 7p 15.3-p 15.1 and consists of 1398 nucleotides that codes for 465 amino acids. The other genes associated with non-syndromic PNDM are Potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11) also known as Kir-6.2 (~30% of PNDM), ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8) also known as SUR1 (~19%), Insulin (INS) (~20%) and pancreatic and duodenal home box 1 (PDX1) (<1 %). KEGG database was used to find out mutated gene responsible for PNDM. The functional interactional partners of GCK were studied using string database, GCK protein is functionally associated with. Glucokinase regulator (GCKR; 2p23), Glucose phosphate isomerase (GPI; 19q13.1), Pyruvate kinase, liver and RBC (PKLR; 1q21), Pyruvate kinase, muscle (PKM2; 1q21) and Insulin (INS; 11p15.5). INS decreases blood glucose concentration by activating GCK directly. GCK mutations indirectly affect pyruvate kinase (PYK) and reduced synthesis of ATP. In the absence of ATP, sulfonylurea receptor 1 (SUR1) in cell membrane of  $\beta$ -cells, causes efflux of the potassium ions due to non-closure of potassium channel. As a result, the potential difference across the membrane becomes more negative. This change in potential difference closes the voltage-gated calcium channels and calcium ions cannot diffuse in  $\beta$ -cells leading to failure of insulin containing vesicles to get fused with the cell surface membrane which causes reduced insulin secretion from  $\beta$ -cells and impaired blood sugar.



control. Consequently GCK mutations indirectly affect associated proteins and persuade hyperglycemia that leads to PNDM.

### **FUNGAL INFECTION IN FANTAIL, A VARIETY OF GOLD FISH, *CARRASIUS AURATUS* L.**

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The aim of present study was to investigate fungal infection in a fantail fish, a variety of goldfish, *Carrasius auratus*. The fish was obtained from pet shop of Lahore. Thirteen fishes were examined from July to October, 2013. Out of 30 fishes 66% fungal infection observed in fishes. The mean total length (TL) and body weight (BW) of fish (n=30) was 9.3cm and 14.9g respectively. The infected fish had ruptured dorsal fins, lesions on skin and caudal peduncle, and eroded anal fin. Fungi isolated from diseased parts of the fish were inoculated on two culture media; Malt extract agar and Sabouraud dextrose agar. The inoculated agar plates were incubated for 5-7 days at 28±2°C. Six fungal genera; *Aspergillus* sp. (100%), *Alternaria* sp. (10%), *Penicillium* sp. (3.3%), *Mucor* sp. (10%), *Rhizopus* sp. (6.6%) and *Fusarium* sp. (3.3%) were isolated from the fish. Gills (12.6%) and abdomen (14.6%) were most affected areas followed by abdomen (14.6%) and head (6.0%). Fins of fishes also show the variable infection; dorsal fin (12%), caudal fin (13.3%), pectoral fin (13.0%), pelvic fin (8.6%) and anal fin shows 10.6% infection. Pure culture plates produced uniform colonies of same colour, indicating the growth of one type of fungus. This study has shown that imported ornamental fish, fantail is having fungal infection. There is an urgent need to control the import of infected and diseased fishes into Pakistan. If this practice continues, there is a risk of spreading fungal infection in local fish species and moreover, it may affect the emerging pet fish industry in Pakistan. Strict regulations on import of ornamental fishes should be implemented at the landing sites in the country.

### **GLIMPSIS OF SCIENTIFIC SIGNS IN THE GLORIOUS QURAN**

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كِتَابُ أَنْزَلْنَاهُ إِلَيْكَ مُبَارَكٌ لِيَدَّبَّرُوا آيَاتِهِ وَلِيَتَذَكَّرَ أُولُو الْأَلْبَابِ

Qura'n is the last Divine book and therefore, contains glimpses of established scientific signs and other statements (verses) that will be discovered over time. The Qura'n exhorts its reader and specifically the believers that the pursuit of exploring this

universe is one of their religious duties that will guide them to the existence of One God. There is no reason to believe that Qura'n has finally yielded all of its scientific secrets and as we enter the next generation we should be confident that Qura'n will continue to shed light on many of the most enduring and intriguing scientific mysteries of our future times. We all have to ponder upon them today and search for what Qura'n has foretold us. As we are educated over time, we see that there are hundreds of scientific signs still waiting for discoveries by the scientists. The session will focus on some of those secrets Qura'n has foretold us and still waiting to be explored such as the reality of information stored in the body cells and their retrieval on the Day of Judgment, the role of DNA and genes in the storage system for human deeds, and its role in testimony and record of actions (deeds)?, the heart secrets, how they are stored and how Allah knows all of these?, the role of human heart in devotion, memorization and comprehension. The electricity of touch during hand shaking and its effect on human personality. Why are hearing, sight and even heart accountable? [Qura'n 17:36]. The book of deeds; is it a written book or something within the human body or both for record of actions? [Qura'n 17:13-14]. The reality of Dabatul Ard?, how it will identify the people? [Qura'n 27: 82]. The geocentric position of mankind, who is more superior to human being?. [Qura'n 17:70]. Accountability, the hidden secrets in human heart and human free will. The spiritual enlightenment and radiating faces . [Qura'n 80: 38-41 and 57:12-15]. Are we alone on this earth or there other extraterrestrial beings (Aliens)? [Qura'n 19:93-95, 27:65 and 42:29]. How is the universe expanded? [Qura'n 51:47, 14: 48, 21: 14, 65:12]. How each and every thing (both living and nonliving) praises and glorifies Allah? (Qura'n 16: 48-50, 17:44, 22: 17-18 and many more verses). The mind of matter, particles, atoms, and its capacity in learning the events and obeying the same God. How is the sustenance of all living beings fulfilled from the Heaven? [Qura'n 11:6]. The soul and its destination [Qura'n 56:83-95]. All these facts will be explained in the light of Qura'n and references therein.

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