

PROCEEDINGS

OF

PAKISTAN CONGRESS OF ZOOLOGY

Volume 39, 2019

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



THIRTY NINTH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

**DEPARTMENT OF ZOOLOGY,
ISLAMIA COLLEGE UNIVERSITY, PESHAWAR**

MARCH 4-6, 2019

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PROCEEDINGS OF THE CONGRESS

Editor

Prof. Dr. A.R. Shakoori

Composed & Designed by: Fakhar Mahmood Shahid

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ACKNOWLEDGMENTS

Department of Zoology, Islamia College University, Peshawar hosted the 39th Pakistan Congress of Zoology (International).

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

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

39th PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL) ISLAMIA COLLEGE UNIVERSITY, PESHAWAR. March 4-6, 2019

DAY ONE**MONDAY, MARCH 04, 2019**

- 08:30 REGISTRATION
10:00 Inauguration: Recitation from the Holy Quran
10:05 Welcome Address by the Vice Chancellor, Islamia College University, Peshawar
10:15 Address by the President, Zoological Society of Pakistan
10:25 Distribution of Medals and Awards
10:45 Address by the Chief Guest
11:15 Vote of Thanks by the Chairman, Department of Zoology, Islamia College University, Peshawar
11:25 Refreshment

HALL-1**12:00 PM-JOINT SESSION I: PLENARY LECTURES****CHAIRPERSON: PROF. DR. A.R. SHAKOORI****CO-CHAIRPERSON: PROF. DR. ALI MUHAMMAD YOUSAFZAI**

1. **Prof. Dr. Norberto Cysne Coimbra**, *Riberirao Preto School of Medicine, University of Sao Paulo, Sao Paulo, Brazil.*
"ENDOGENOUS OPIOID PEPTIDES MODULATE PANIC-LIKE EMOTIONS: INTERACTIONS BETWEEN OPIOID AND GABAERGIC SYSTEMS"
2. **Dr. Muhammad Ibrahim**, *Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar*
"ZEBRAFISH: A VERSATILE MODEL FOR IN VIVO AND IN VITRO STUDIES"

1:30 - 2:30 PM LUNCH AND PRAYER BREAK (ZUHAR)**2:30 - 4:15 PM****HALL-1****HALL-2**
SECTION I
**CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY,
GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY**
SESSION I
Chairman: Dr. Farah Rauf Shakoori**Co-Chairperson: Dr. Hafiz Abdullah Sakir**

- | | | |
|-----|--------------------------------|----------|
| 1. | I. Liaqat (GCU, Lahore) | CBGP-99 |
| 2. | A. Khan (KUST) | CBGP-100 |
| 3. | A.A. Latif (LCWU, Lahore) | CBGP-101 |
| 4. | S. Andleeb (UAIK, Muzafarabad) | CBGP-102 |
| 5. | S. Rehman (GCU, Lahore) | CBGP-103 |
| 6. | S. Saeed (UP, Lahore) | CBGP-104 |
| 7. | A. Anwar (UP, Lahore) | CBGP-105 |
| 8. | A. Rab (KUST, Kohat) | CBGP-106 |
| 9. | N. Ullah (U Swat) | CBGP-107 |
| 10. | R. Ishaq (GCWU, Sialkot) | CBGP-108 |
| 11. | M.T Zahid (GCU, Lahore) | CBGP-109 |

SECTION II
PEST AND PEST CONTROL
SESSION I
Chairman: Abdul Aziz Khan**Co-Chairperson: Dr. Abdul Manan Shaikh**

- | | | |
|-----|-----------------------------|-------|
| 1. | W.A Naveed (FAFU, China) | PC 45 |
| 2. | M. Saeed (MNSUA, Multan) | PC 46 |
| 3. | G.Z Khan (NIFA, Peshawar) | PC 47 |
| 4. | U. Ahmed (MNSVA, Multa) | PC 48 |
| 5. | T. Maqsood (UA, Faisalabad) | PC 49 |
| 6. | M. Ramzan (MNSUA, Multan) | PC 50 |
| 7. | N. Mehmood (US, Sargodha) | PC 51 |
| 8. | I. Anwar (MNSUA, Multan) | PC 52 |
| 9. | H. Abbas (MNSUA, Multan) | PC 53 |
| 10. | Q. Ali (MNSUA, Multan) | PC 54 |

2:30 - 4:15 PM		
HALL-3		HALL-4
SECTION IV PARASITOLOGY SESSION 1 Chairman: Prof. Dr. Syed Akram Shah Co-Chairperson: Prof. Dr. Mian Sayed Khan		SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 2 Chairman: Dr. Soumble Zulfiqar Co-Chairperson: Dr. Farhad Ullah
1.	H. Soofi (US, Jamshoro)	PAR-1
2.	A. Khan (UK, Karachi)	PAR-2
3.	S.A Buriro (US, Jamshoro)	PAR-3
4.	A. Abdullah (SAU, Tandojam)	PAR-4
5.	H.M. Solangi (SAU, Tandojam)	PAR-5
6.	H. Gul (SALU, Khairpur)	PAR-6
7.	Suleman (CAAS, China)	PAR-7
8.	M.S. Khan (Uswabi, KPK)	PAR-8
9.	I. Chandio (US, Jamshoro)	PAR-9
10.	R. Hadi (JUW, Karachi)	PAR-10
11.		
1.	I. Ullah (UA, Faisalabad)	CBGP-143
2.	M.F. Siddiqui (QAU, Islamabad)	CBGP-144
3.	K.Z. Lodhi (QAU, Islamabad)	CBGP-145
4.	J.A. Khokher (US, Jamshoro)	CBGP-146
5.	R. Ejaz (SBBWU, Peshawar)	CBGP-147
6.	G. Shaheen (QAU, Islamabad)	CBGP-148
7.	M. David (QAU, Islamabad)	CBGP-149
8.	H. Joyo (US, Jamshoro)	CBGP-150
9.	R. Mushtaq (FUUAST)	CBGP-151
10.	N. Shah (US, Jamshoro)	CBGP-152
11.	I. Maqsood (SBBWU, Peshawar)	CBGP-153
	S. Mahin (WU, Multan)	CBGP-154
HALL-5		
SECTION V FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY SESSION 1 Chairman: Prof. Dr. Aleem Ahmed Khan Co-Chairperson: Dr. Muhammad Rais		
1.	B. Soomro (US, Jamshoro)	FEWFM-70
2.	Z.I.K. Bhatti (BRC, Islamabad)	FEWFM-71
3.	A.U.H. Faiz (WU, Azad Kashmir)	FEWFM-72
4.	Z.U.R. Awan (PGC, Bannu)	FEWFM-73
5.	S. Khalil (IUB, Bhawalpur)	FEWFM-74
6.	R. Athar (IUB, Bhawalpur)	FEWFM-75
7.	T. Mahmood (AAU, Rawalpindi)	FEWFM-76
8.	S. Ali (AAU, Rawalpindi)	FEWFM-77
9.	L.U Khan (AAU, Rawalpindi)	FEWFM-78
10.	G. H. Brohi (GDC, Nasirabad)	FEWFM-79
11.	B.N. Khan (UP, Lahore)	FEWFM-80
12.	Z.U. Raees (HU, Mansehra)	FEWFM-81
13.	S. Afzal (UOL, Lahore)	FEWFM-82
14.	S. Khalid (UOL, Lahore)	FEWFM-83
15.	S. Ashraf (UOL, Lahore)	FEWFM-84

4:15 - 4:30 PM		TEA BREAK AND PRAYER BREAK (ASAR)	
4:30 - 5:45 PM			
HALL-1		HALL-2	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 2 Chairman: Prof. Dr. Khalil Ahmad Co-Chairperson: Dr. Abdul Rehman		SECTION II PEST AND PEST CONTROL SESSION 2 Chairman: Prof. Dr. Muhammad Saeed Wagan Co-Chairperson: Dr. Abida Butt	
1. A. Irfan (UP, Lahore)	CBGP-110	1. M.A. Rafique (MNSUA, Multan)	PC-55
2. R. Mushtaq (GCU, Lahore)	CBGP-111	2. S. Saeed (MNSUA, Multan)	PC-56
3. F. Shafiq (MUST, Mirpur)	CBGP-112	3. M.Z. Majeed (US, Sargodha)	PC-57
4. I. Ullah (KUST, Kohat)	CBGP-113	4. M.N. Khan (MNSUA, Multan)	PC-58
5. S. Saleemi (UP, Lahore)	CBGP-114	5. A. Feroz (UP, Lahore)	PC-59
6. W. Ullah (KUST, Kohat)	CBGP-115	6. M.F. Qaiser (MNSUA, Multan)	PC-60
7. A. Amin (UCP, Lahore)	CBGP-116	7. M. Ghous (MNSUA, Multan)	PC-61
8. S. Shoukat (UCP, Lahore)	CBGP-117		
9. F. Tariq (UCP, Lahore)	CBGP-118		
10. A. Aslam (UCP, Lahore)	CBGP-119		
11. I. Shahid (UCP, Lahore)	CBGP-120		
12. J.I. Wattoo (UCP, Lahore)	CBGP-121		
13. M. Naveed (UCP, Lahore)	CBGP-122		
14. F. Jabeen (UCP, Lahore)	CBGP-123		
15. S. Abbas (NARC, Islamabad)	CBGP-124		
16. S. Gul (U Balochistan, Quetta)	CBGP-125		
HALL-3		HALL-4	
SECTION IV PARASITOLOGY SESSION 2 Chairman: Dr. Aly Khan Co-Chairperson: Dr. Saima Naz		SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 3 Chairman: Dr. Asia Bibi Co-Chairperson: Dr. Saba Irshad	
1. N. Nisa (VPCI, Karachi)	PAR-11	1. S. Ullah (U Swabi)	CBGP-155
2. M. Ijaz (UVAS, Lahore)	PAR-12	2. F. Jabeen (U Swabi)	CBGP-156
3. A.M. Jakhriani (US, Jamshoro)	PAR-13	3. S. Awan (UAJK, Muzaffarabad)	CBGP-157
4. Sidiqat-ul-Kubra (US, Jamshoro)	PAR-14	4. B. Uddin (U Swabi)	CBGP-158
5. S. Anwer (US, Jamshoro)	PAR-15	5. R. Musadique (GCU, Lahore)	CBGP-159
6. S. Soomro (US, Jamshoro)	PAR-16	6. R.A. Soomro (SAU, Tandojam)	CBGP-160
7. S.N. Das (US, Jamshoro)	PAR-17	7. R. Abaidullah (GCU, Lahore)	CBGP-161
8. A.S. Hulio (US, Jamshoro)	PAR-18	8. A. Bibi (WU, Mardan)	CBGP-162
		9. I. Naureen (Minhaj U, Lahore)	CBGP-163
		10. N. Younus (QAU, Islamabad)	CBGP-164

4:15 - 4:30 PM			
HALL-5			
SECTION V FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY SESSION 2 Chairman: <i>Dr. Abdul Qadir</i> Co-Chairperson: <i>Dr. Abdul Rahim</i>			
1.	W. Ali (LU, Uthal)	FEWFM-32	
2.	S. Hussain (U Swabi)	FEWFM-33	
3.	W.A. Shafrani (US Jamshoro)	FEWFM-34	
4.	A.N. Soomro (US Jamshoro)	FEWFM-35	
5.	N. Sial (IUB, Bahawalpur)	FEWFM-36	
6.	M.A. Gondal (COMSATS U, Islamabad)	FEWFM-37	
7.	A. Rahim (U Malakand)	FEWFM-38	
8.	M. Ali (UA, Faisalabad)	FEWFM-1	
9.	M.Z.E. Arif (UA, Faisalabad)	FEWFM-2	
10.	F. Bari (QAU, Islamabad)	FEWFM-3	
11.	U. Ramzan (UA, Faisalabad)	FEWFM-4	
12.	R. Chandio (DUHS, Karachi)	FEWFM-5	
5:45 - 6:00 PM PRAYER BREAK (MAGHRIB)			
6:00 - 7:45 PM			
HALL-1		HALL-2	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 4 Chairman: <i>Prof. Dr. Javed Iqbal Qazi</i> Co-Chairperson: <i>Dr. Rehman Mehmood Khattak</i>		SECTION II PEST AND PEST CONTROL SESSION 3 Chairman: <i>Prof. Dr. Nasreen Memoon</i> Co-Chairperson: <i>Dr. Unsar Naeem Ullah</i>	
1.	M. Shaikh (US, Jamshoro)	CBGP-75	1. I.H. Malana (MNSUA, Multan) PC-68
2.	H. Sajjad (US, Jamshoro)	CBGP-76	2. A. Riaz (NIAB, Faisalabad) PC-62
3.	A. Anwaar (Minhaj U, Lahore)	CBGP-77	3. A. Siddique (MNSUA, Multan) PC-63
4.	S.M.H. Malik (US, Jamshoro)	CBGP-78	4. S. Sharif (IUB, Bahawalpur) PC-64
5.	J. Rehman (Usawabi)	CBGP-79	5. M. Nasir (PAD, Lahore) PC-65
6.	A. Masih (UJAK, Muzaffarabad)	CBGP-80	6. M.A. Khan (UA, Faisalabad) PC-66
7.	S. Kazmi (AAU, Rawalpindi)	CBGP-81	7. M. Usman (UA, Faisalabad) PC-67
8.	S. Irshad (UP, Lahore)	CBGP-82	8. N. Akhtar (UA, Faisalabad) PC-69
9.	S. Hossain (AAU, Rawalpindi)	CBGP-83	9. M. Adnan (MNSUA, Multan) PC-70
10.	I. Gul (KUST, Kohat)	CBGP-84	10. R.A. Ayaz (MNSUA, Multan) PC-71
11.	Aiman (U Swabi)	CBGP-85	11. T. Ahmad (UA, Faisalabad) PC-72
12.	Asghar Abbas (MNSUA, Multan)	CBGP-86	
13.	S.S. Gilani (LDD, Punjab)	CBGP-87	
14.	S. Saleem (SBBWU, Peshawar)	CBGP-88	

6:00 - 7:45 PM					
HALL-3			HALL-4		
SECTION IV PARASITOLOGY SESSION 3			SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 5		
Chairman: Dr. Noor un Nisa Co-Chairperson: Dr. Mushtaq Hussain Lashari			Chairman: Dr. Najma Shaheen Co-Chairperson: Dr. Muhammad Khan		
1.	N.A. Birmani (US, Jamshoro)	PAR-19	1.	A. Hussain (QAU, Islamabad)	CBGP-165
2.	H. Laghari (US, Jamshoro)	PAR-20	2.	M. Ahmad (QAU, Islamaabd)	CBGP-166
3.	F. Ahmed (US, Jamshoro)	PAR-21	3.	K. Tariq (GCWU, Faisalaabd)	CBGP-167
4.	I.A. Kapir (US, Jamshoro)	PAR-22	4.	A. Gul (MNSUA, Multan)	CBGP-168
5.	B. Siyal (US, Jamshoro)	PAR-23	5.	H. Ahmed (WU Swabi)	CBGP-169
6.	M.M. Abro (GC, Hyderabad)	PAR-24	6.	M.W. Ameer (IUB, Bahawalpur)	CBGP-170
7.	N. Khatoon (UK, Karachi)	PAR-25	7.	H. Khan (UA, Peshawar)	CBGP-171
8.	S. Sajjad (UK, Karachi)	PAR-26	8.	F. Bahadar (GCWU, Faisalabad)	CBGP-172
9.	I. Fatima (US, Jamshoro)	PAR-27	9.	F. Bibi (BZU, Multan)	CBGP-173
10.	J. Lohana (US, Jamshoro)	PAR-28	10.	Z.S. Amin (UCP, Lahore)	CBGP-174
11.	S. Naz (US, Jamshoro)	PAR-29	11.	M. Afzal (UCP, Lahore)	CBGP-175
			12.	T. Aqeel (UCP, Lahore)	CBGP-176
			13.	I. Kainat (UCP, Lahore)	CBGP-177
			14.	M. Aslam (UCP, Lahore)	CBGP-178
			15.	S. Riaz (UCP, Lahore)	CBGP-179
HALL-5					
SECTION V FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY SESSION 3					
Chairman: Dr. Tariq Mahmood Co-Chairperson: Dr. Shaista Jalbani					
1.	F. Ahmad (AED)	FEWFM-85			
2.	I. Ansari (PAFIKET, Karachi)	FEWFM-86			
3.	H. Lakho (US, Jamshoro)	FEWFM-87			
4.	N. Ullah (SBBU, Sheringal)	FEWFM-88			
5.	M. Rais (UAA, Rawalpindi)	FEWFM-89			
6.	A. Shahzad (UAA, Rawalpindi)	FEWFM-90			
7.	Z. Khalid (UAA, Rawalpindi)	FEWFM-91			
8.	A. Hussain (UAA, Rawalpindi)	FEWFM-92			
9.	B.N. Khan (UP, Lahore)	FEWFM-93			
10.	M. Azhar (UP, Lahore)	FEWFM-94			
11.	M. Aziz (UAA, Rawalpindi)	FEWFM-95			
12.	M. Araan (US, Jamshoro)	FEWFM-96			
13.	I. Arain (US, Jamshoro)	FEWFM-97			
14.	R.A. Khan (GGDC, Mansehra)	FEWFM-98			
15.	A. Saleem (GGDC, Mansehra)	FEWFM-99			
16.	W.M. Butt (UP, Lahore)	FEWFM-100			
17.	I. Zia (QAU, Islamabad)	FEWFM-101			
8:00 PM					
DINNER					

DAY TWO**TUESDAY, MARCH 05, 2019****HALL-1**

9:00 AM—JOINT SESSION II: PLENARY LECTURES
CHAIRPERSON: PROF. DR. A.R. SHAKOORI
CO-CHAIRPERSON: PROF. DR. NAEEM TARIQ NAREJO

1. **Prof. Dr. Awad Mohamed Awad Hassan**, *Department of Ecological Agriculture & Agri-Environmental Design, Szent Istvan University, Hungary*
“APPLICATION OF CITIZEN SCIENCE, DATE MINING, AND SPATIAL CONCEPTS IN ZOOLOGY AND AGRICULTURE”
2. **Abdul Aziz Khan**, *Secretary General, Zoological Society of Pakistan*
“ZOOLOGICAL SOCIETY OF PAKISTAN – IN PURSUIT OF EXCELLENCE (1968-2018)”

11:00 AM**TEA BREAK****11:30 - 1:00 PM****HALL-1****HALL-2**

SECTION I
CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY,
GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY
SESSION 6

Chairman: *Dr. Asmatullah*
Co-Chairperson: *Dr. Gul Nabi Khan*

SECTION II
PEST AND PEST CONTROL
SESSION 4

Chairman: *Prf. Dr. Naureen Rana*
Co-Chairperson: *Dr. Amjad Farooq*

1. A. Nasir (SBKWU, Quetta) CBGP-126
2. A. Karim (GCS, Wahdat Rd, Lahore) CBGP-127
3. F. Mehmood (Minhaj U, Lahore) CBGP-128
4. F. Kanwal (DHU, Shanghai) CBGP-129
5. F. Hassan (UVAS, Lahore) CBGP-130
6. I. Aziz (UP, Lahore) CBGP-131
7. F. Tong (GUN, Guangxi, China) CBGP-132
8. M. Arshad (IUB, Bahawalpur) CBGP-133
9. R. Adalat (LCWU, Lahore) CBGP-134
10. F. Saleem (LCWU, Lahore) CBGP-135
11. S. Karamat (UAJK, Muzaffarabad) CBGP-136
12. N. Shafi (UAJK, Muzaffarabad) CBGP-137
13. M. Bibi (UAJK, Muzaffarabad) CBGP-138
14. N. Saher (CEMB, Karachi) CBGP-139
15. M. S. Shahzad (UCP, Lahore) CBGP-140
16. R. Fatima (UCP, Lahore) CBGP-141
17. T. Kanwal (GCU, Lahore) CBGP-142

1. M.U. Khokhar (SAU, Tandojam) PC-73
2. G.Q. Junejo (SAU, Tandojam) PC-74
3. M. Nauman (MNSUA, Multan) PC-75
4. M. Rizwan (NIA, Tandojam) PC-76
5. M.U. Asif (NIA, Tandojam) PC-77
6. T. Riaz (UCP, Lahore) PC-78
7. F. Naz (UCP, Lahore) PC-79
8. M.A. Randhawa (NIA, Tandojam) PC-80
9. S.A. Tariq (PARC, Karach) PC-81
10. S. Siddiqui (SAU, Tandojam) PC-82
11. M. Nawaz (JUW, Karachi) PC-83
12. M.U. Shahzad (MNSUA, Multan) PC-84
13. M. Yasir (UA, Faisalabad) PC-85
14. N. Ahmed (UP, Lahore) PC-86
15. A. Aihetsham (UP, Lahore) PC-87
16. Qurat-ul-Ain (UP, Lahore) PC-88

11:30 - 1:00 PM			
HALL-3		HALL-4	
SECTION IV PARASITOLOGY SESSION 4		SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 6	
Chairman: <i>Prof. Dr. Juma Khan Kakar</i> Co-Chairperson: <i>Dr. Ijaz Ali</i>		Chairman: <i>Dr. Shahid Nadeem</i> Co-Chairperson: <i>Dr. Muhammad Tariq Zahid</i>	
1. R. Chandio (UK, Karachi)	PAR-30	1. Q. A. Memon (US, Jamshoro)	CBGP 180
2. S. Hingoro (US, Jamshoro)	PAR-31	2. M.A. Khan (UA, Faisalabad)	CBGP 181
3. S. Khaskheli (US, Jamshoro)	PAR-32	3. M. Bhanwrio (US, Jamshoro)	CBGP 182
4. S. Begum (US, Jamshoro)	PAR-33	4. Z.A. Laghari (US, Jamshoro)	CBGP 183
5. Y. Kharik (US, Jamshoro)	PAR-34	5. A.Hussain (UAJK, Kotli)	CBGP 185
6. M.H. Lashari (IUB)	PAR-35	6. L.A. Solangi (US, Jamshoro)	CBGP 186
7. M.Z. Kayani (GBP, DA, Rawalpindi)	PAR-36	7. K. Hayat (MNSUA, Multan)	CBGP 45
8. T. Mukhtar (AAU, Rawalpindi)	PAR-37	8. S. Arbab (MNSUA, Multan)	CBGP 46
9. A.A. Khan (AWKU, Mardan)	PAR-38	9. A. Sarwar (UP, Lahore)	CBGP 47
10. S. Ullah (U Swabi)	PAR-39	10. M. Arshad (U Sargodha)	CBGP 48
11. S. Ullah (UK, Karachi)	PAR-40	11. M.A. Nawaz (SBBU, Sheringal)	CBGP 49
		12. F. Farah (JUW, Karachi)	CBGP 50
		13. S. Rasheed (UP, Lahore)	CBGP 51
		14. K.H. Lashari (US, Jamshoro)	CBGP 52
HALL-5			
SECTION V FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY SESSION 4			
Chairman: <i>Prof. Dr. Quddusi B. Kazmi</i> Co-Chairperson: <i>Dr. Khalid Usman</i>			
1. Z.A Pir (LCI, India)	FEWFM-102		
2. K. Niaz (WU, AJK)	FEWFM-103		
3. A.H. Rana (IUB)	FEWFM-104		
4. A. Rahman (GPGC, Buzmu)	FEWFM-105		
5. G. Khan (UOL, Lahore)	FEWFM-106		
6. Y. Karim (U Swabi)	FEWFM-107		
7. G.S. Gachal (US Jamshoro)	FEWFM-108		
8. I. Tagar (US Jamshoro)	FEWFM-109		
9. W.M. Zaheer (UK, Karachi)	FEWFM-110		
10. S. Hussain (QAU, Islamabad)	FEWFM-111		
11. Q.B. Kazmi (UK, Karachi)	FEWFM-112		
12. S. Kanwal (UA, Faisalabad)	FEWFM-113		
13. B. Ansari (GCU, Faisalabad)	FEWFM-114		
14. J. Altuf (GCU, Faisalabad)	FEWFM-115		
15. S. Nasrullah (SCW, Sialkot)	FEWFM-116		

1:30 - 2:30 PM		LUNCH AND PRAYER BREAK (ZUHAR)	
2:30 - 4:15 PM			
HALL-1		HALL-2	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 7		SECTION III ENTOMOLOGY SESSION 1	
Chairman: <i>Prof. Dr. Nuzhat Shafi</i> Co-Chairperson: <i>Dr. Shagufta Naz</i>		Chairman: <i>Dr. Riffat Sultana</i> Co-Chairperson: <i>Dr. Waheed Ali Panhwar</i>	
1. S. Naz (LCWU, Lahore)	CBGP - 53	1. N.A. Shah (US, Jamshoro)	ENT - 1
2. A. Kanwal (UP, Lahore)	CBGP - 54	2. A.R.Khan (LC, Indne)	ENT - 2
3. G. Ali (UAJK, Muzaffarabad)	CBGP - 55	3. S. Mangi (SALU, Khairpur)	ENT - 3
4. A.A. Abbasi (MUST, Mirpur)	CBGP - 56	4. A. Raza (US Jamshoro)	ENT - 4
5. J.Alam (MUST, Mirpur)	CBGP - 57	5. I.A. Channa (US, Jamshoro)	ENT - 5
6. T.A. Mughal (MUST, Mirpur)	CBGP - 58	6. K.A. Shar (SALU, Khairpur)	ENT - 6
7. N. Tariq (UA Faisalabad)	CBGP - 59	7. W.A. Panhwar (SALU, Khairpur)	ENT - 7
8. F. Ambreen (GCW, Faisalabad)	CBGP - 60	8. H. Bhatti (SALU, Khairpur)	ENT - 8
9. H. Mahreen (WU, Multan)	CBGP - 61	9. S.T. Muntha (US, Jamshoro)	ENT - 9
10. P.A. Ali (WU, Swabi)	CBGP - 62	10. J. Shaikh (US, Jamshoro)	ENT - 10
11. M. Faheem (QAU, Islamabad)	CBGP - 63	11. F. Shaikh (US, Jamshoro)	ENT - 11
12. M. Muzammil (Gomal U, D.I.Khan)	CBGP - 64	12. K. Saeed (U Buner)	ENT - 12
13. A. Masood (UCP, Lahore)	CBGP - 65	13. A.R. Soomro (US, Jamshoro)	ENT - 13
14. N.J. Begum (KUST, Kohat)	CBGP - 66	14. N. Memon (US, Jamshoro)	ENT - 14
15. A. Siddique (UP, Lahore)	CBGP - 67	15. P. Soomro (SALU, Khairpur)	ENT - 15
16. A. Riaz (GCU, Faisalabad)	CBGP - 68	16. A.N. Memnr (GDC, Dadu)	ENT - 16
17. M. Ali (QAU, Islamabad)	CBGP - 69	17. R. Sultana (US, Jamshoro)	ENT - 17
18. K. Javed (KUST, Kohat)	CBGP - 70	18. B.A. Bughio (US, Jamshoro)	ENT - 18
19. M. Rafi (PIEAS, Islamabad)	CBGP - 71		
20. A. Imtiaz (UP, Lahore)	CBGP - 72		
21. M. Iqbal (GCU, Lahore)	CBGP - 73		
22. A.R. Abbasi (US, Jamshoro)	CBGP - 74		
HALL-3			
SECTION IV PARASITOLOGY SESSION 5			
Chairman: <i>Prof. Dr. Asmatullah Kakar</i> Co-Chairperson: <i>Dr. Asma Waheed Qureshi</i>			
1. J. Bashir (UK, Karachi)	PAR-41	11. A. Sattar (UK, Karachi)	PAR- 52
2. S. Fayyaz (WU, AJK)	PAR-42	12. A. Khan (U Peshawar)	PAR- 53
3. S. Tariq (US, Jamshoro)	PAR-43	13. AW Qureshi (GCWU, Sialkot)	PAR- 54
4. M. Iqbal (HU, Mansehra)	PAR-46	14. Rohullah (VU, Lahore)	PAR- 55
5. I. Khan (HU, Mansehra)	PAR-45	15. O.O. Amioov (UAS, Tashkent, Uzbekistan)	PAR- 56
6. Z. Butt (US, Jamshoro)	PAR-47	16. A.E. Kuchboev (UAS, Tashkent, Uzbekistan)	PAR- 57
7. N. H. Khuro (NIA, Tandojam)	PAR-48	17. Z. Akram (MUST, AJK)	PAR- 59
8. T. Khan (UM, Chakdara, L.Dir)	PAR-49	18. A.Kakar (UB, Quetta)	PAR- 61
9. N. Khan (ICU, Peshawar)	PAR-50		
10. W. Khan (U Malakand, L.Dir)	PAR-51		

2:30 - 4:15 PM		
HALL-4		HALL-5
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 10		SECTION III ENTOMOLOGY SESSION 2
Chairman: <i>Prof. Ali Muhammad Yousafzai</i> Co-Chairperson: <i>Dr. Muzammil Ahmad Khan</i>		Chairman: <i>Dr. Muhammad Ather Rafi</i> Co-Chairperson: <i>Dr. M. Momoon-ur-Rashid</i>
1. M. Nisa (UA, Faisalabad) CBGP- 187		1. M.R. Pitazi (US, Jamsoro) ENT- 19
2. U. Nazir (UA, Faisalabad) CBGP- 188		2. F. Haider (US, Jamsoro) ENT- 20
3. A. Razzaq (UA, Faisalabad) CBGP- 189		3. M.J. Bozdar (US, Jamsoro) ENT- 21
4. H.Naz (CUVAS, Bahawalpur) CBGP- 190		4. M.A. Jakhani (US, Jamsoro) ENT- 22
5. M.Z.Hakro (US Jamsoro) CBGP- 191		5. M. Jamil (US, Jamsoro) ENT- 23
6. N.Shoaib (UK Karachi) CBGP- 192		6. S. Soomro (US, Jamsoro) ENT- 24
7. R. Naz (UA, Faisalabad) CBGP- 193		7. A.A. Afghan (US, Jamsoro) ENT- 25
8. M. Arshad (UP, Lahore) CBGP- 194		8. A. Chandio (US, Jamsoro) ENT- 26
9. C.Ara (UP, Lahore) CBGP- 195		9. M.A Pitafi (US, Jamsoro) ENT- 27
10. S. Ali (GCU, Lahore) CBGP- 196		10. F.D Soomro (US, Jamsoro) ENT- 28
11. S. Mumtaz (UAJK, Muzaffarabad) CBGP- 197		11. M. Lal (US, Jamsoro) ENT- 29
12. M.A. Latif (GCU, Faisalabad) CBGP- 198		12. N. Bhanger (US, Jamsoro) ENT- 30
13. H. Afzal (MNSUA, Multan) CBGP- 199		13. S. Fatimah (US, Jamsoro) ENT- 31
14. S. Bashir (MNSUA, Multan) CBGP- 200		14. M. Noman (US, Jamsoro) ENT- 32
15. B. Bhatti (UA, Faisalabad) CBGP- 201		15. N.Soomro (US, Jamsoro) ENT- 33
16. M. Noreen (LCWU, Lahore) CBGP- 202		16. S. Soomro (US, Jamsoro) ENT- 34
17. S.Khawaja (FUUAST, Karachi) CBGP- 203		
18. A. Zahid (Ningbo U, China) CBGP- 204		
4:15 - 4:30 PM TEA BREAK AND PRAYER BREAK (ASAR)		
4:30 - 5:45 PM		
HALL-1		HALL-2
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 9		SECTION V FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY SESSION 5
Chairman: <i>Dr. Dil Ara Abbass Bukhari</i> Co-Chairperson: <i>Dr. Nadeem Sheikh</i>		Chairman: <i>Prof. Dr. M. Afzal Kazmi</i> Co-Chairperson: <i>Dr. Azra Bano</i>
1. K. Akhrar (GPGC, Karak) CBGP - 89		1. M. Mehwish (JUW, Karachi) FEWFM- 39
2. H.M. Arsalan (UCP, Lahore) CBGP - 90		2. T. Hamid (UK, Karachi) FEWFM- 40
3. S. Mansoor (UCP, Lahore) CBGP - 91		3. H. Moin (JUW, Karachi) FEWFM- 41
4. H. Mubeen (UCP, Lahore) CBGP - 92		4. V.A. Qadir (UK, Karachi) FEWFM- 42
5. A. Aslam (UCP, Lahore) CBGP - 93		5. M.A. Mahar (US, Thatta) FEWFM- 43
6. Z.Nadeem (UCP, Lahore) CBGP - 94		6. U. Aziz (APWA College, Karachi) FEWFM- 44
7. S.Raza (UCP, Lahore) CBGP - 95		7. A.H.Narejo (UK, Karachi) FEWFM- 45
8. S.Kanwal (COMSATS, Sahiwal) CBGP - 96		8. S.H. Noor (UK, Karachi) FEWFM- 46
9. R. Ahmad (GKKSTH, Lahore) CBGP - 97		9. F. Naz (UK, Karachi) FEWFM- 47
10. M. Zafer (U.Gujrat) CBGP - 98		10. M. Rodini (LUAWMS, Balochistan) FEWFM- 48
11. S. Afzal (KUST, Kohat) CBGP - 236		11. F. Iqbal (UK, Karachi) FEWFM- 49
12. N. Ullah (KUST, Kohat) CBGP - 237		12. A. Bano (LUAWMS, Balochistan) FEWFM- 50
13. S. Ullah (KUST, Kohat) CBGP - 238		13. M.E. Ullah (LUAWMS, Balochistan) FEWFM- 51
14. Z.A. Laghari (CAS, China) CBGP - 239		14. N.A. Tamimi (UITU, Karachi) FEWFM- 52
15. Z. Naz (NIBGE, Faisalabad) CBGP - 240		15. S. Odhano (UK, Karachi) FEWFM- 53
16. S. Sba (MNSUA, Multan) CBGP - 241		
17. A. Iqbal (UP, Lahore) CPGP - 242		

4:30 - 5:45 PM					
HALL-3			HALL-4		
SECTION II PEST AND PEST CONTROL SESSION 6 Chairman: Prof. Dr. Naheed Baloch Co-Chairperson: Prof. Dr. Sohail Ahmad			SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 9 Chairman: Dr. Chaman Ara Co-Chairperson: Dr. Humayun Khan		
1.	N. Baloch (US, Sjamshoro)	PC- 1	1.	A. Iqbal (UVAS, Lahore)	CBGP - 205
2.	A.A. Khan (BZU, Layyah)	PC- 2	2.	Z. Liaquat (UA, Faisalabad)	CBGP - 206
3.	H. Afzal (UA, Faisalabad)	PC- 3	3.	R. Ilyas (GCWU, Faisalabad)	CBGP - 207
4.	N. Ahmed (UA, Faisalabad)	PC- 4	4.	A. Alam (UA, Faisalabad)	CBGP - 208
5.	S. Kashaf (UA, Faisalabad)	PC- 5	5.	M.S.Haider (UOL, Lahore)	CBGP - 209
6.	H. Sadiq (UA, Faisalabad)	PC- 6	6.	S. Liaquat (UOL, Lahore)	CBGP - 210
7.	M.Z.Khalid (UA, Faisalabad)	PC- 7	7.	Z. Bashir (UA, Faisalabad)	CBGP - 211
8.	Z. Fatima (UA, Faisalabad)	PC- 8	8.	N. Saba (UOL, Sargodha)	CBGP - 212
9.	H. Zahid (UA, Faisalabad)	PC- 9	9.	A. Munir (UOL, Lahore)	CBGP - 213
10.	N.J.Gill (UA, Faisalabad)	PC- 10	10.	I. Anwar (MNSUA, Multan)	CBGP - 214
			11.	Z.S.Khan (U Swabi)	CBGP - 215
HALL-5					
SECTION III ENTOMOLOGY SESSION 3 Chairman: Dr. Gul Zamin Khan Co-Chairperson: Dr. Muhammad Zeeshan Majeed					
1.	S. Fatimah (US, Jamshoro)	ENT-35			
2.	S.A. Larik (US Jamshoro)	ENT-36			
3.	S. Sanam (US, Jamshoro)	ENT-37			
4.	V. Kumari (US Jamshoro)	ENT-38			
5.	Z. Abbasi (US, Jamshoro)	ENT-39			
6.	M.M. Ayaz (BZU, Multan)	ENT-40			
7.	S. Hussain (U Swabi)	ENT-41			
8.	S. Sanam (US, Jamshoro)	ENT-42			
9.	J.K. Turk (US, Jamshoro)	ENT-43			
10.	A.U. Rehman (U Swabi)	ENT-44			
11.	I.A. Magasi (SAU, Tandojam)	ENT-46			
12.	R. Sahito (US, Jamshoro)	ENT-45			
13.	S. Ghafoor (MNSUA, Multan)	ENT-47			
14.	A.Babar (MNSUA, Multan)	ENT-48			
15.	S.U. Zameer (MNSUA, Multan)	ENT-49			
16.	M.S. Wagan (US, Jamshoro)	ENT-50			
5:45 - 6:00 PM			PRAYER BREAK (MAGHRIB)		

6:00 - 7:45 PM			
HALL-1		HALL-2	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 10 Chairman: Prof. Dr. Nusrat Jahan Co-Chairperson: Dr. Muhammad Zahid		SECTION V FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY SESSION 6 Chairman: Dr. Muhammad Akbar Khan Co-Chairperson: Dr. Abdul Majid	
1. Baboo (CUBAS, Bahawalpur)	CBGP-1	1. K. Aftab (UG, Gujrat)	FEWFM- 54
2. D.A. Solangi (US, Jamshoro)	CBGP-2	2. M. Hanif (UA, Faisalabad)	FEWFM- 55
3. T. Hussain (Minhaj U, Lahore)	CBGP-3	3. M.A. Babar (UP, Lahore)	FEWFM- 56
4. A. Nawaz (LCWU, Lahore)	CBGP-4	4. S.G. Abbas (UP, Lahore)	FEWFM- 57
5. H. Hamid (LCWU, Lahore)	CBGP-5	5. M.T. Waseem (UP, Lahore)	FEWFM- 58
6. R. Chaudhary (PCSIR, Islamabad)	CBGP-6	6. M.A. Khan (UP, Lahore)	FEWFM- 59
7. M.T. Chaudhary (PCSIR, Islamabad)	CBGP-7	7. M. Asim (UP, Lahore)	FEWFM- 60
8. K.J. Iqbal (IUB, Bahawalpur)	CBGP-8	8. K. Sultana, (UP, Lahore)	FEWFM- 61
9. H. Kalhoro (US, Jamshoro)	CBGP-9	9. M.K. Nawaz (UP, Lahore)	FEWFM- 62
10. H. Bibi (KUST, Kohat)	CBGP-10	10. K. Mahmood (UP, Lahore)	FEWFM- 63
11. L. Bibi (KUST, Kohat)	CBGP-11	11. Mamoon (GCU, Faisalabad)	FEWFM- 64
12. R. Abdullah (LCWU, Lahore)	CBGP-12	12. R.M. Ahmad (RC, Nankana Sahib)	FEWFM- 65
13. A. Kaleem (LCWU, Lahore)	CBGP-13	13. A.M. Khan (UP, Lahore)	FEWFM- 66
14. M. Iqtedar (LCWU, Lahore)	CBGP-15	14. A. Rafiq (UP, Lahore)	FEWFM- 67
15. B. Alya (LCWU, Lahore)	CBGP-14	15. A. Iqbal (UP, Lahore)	FEWFM- 68
		16. S. Aslam (UP, Lahore)	FEWFM- 69
		17. R.M. Tariq (UOK, Karachi)	GZ-1
HALL-3		HALL-4	
SECTION II PEST AND PEST CONTROL SESSION 7 Chairman: Dr. Hafiz Muhammad Tahir Co-Chairperson: Dr. Tanzeela Riaz		SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION II Chairman: Dr. Basit Zeeshan Co-Chairperson: Dr. Muhammad Attaullah	
1. S. Mangi (US, Jamshoro)	PC- 11	1. W. Ahmand (USwabi)	CBGP-216
2. S. Kumar (CUBAS, Bahawalpur)	PC- 12	2. I.U. Rahman (USwabi)	CBGP-217
3. A.A. Samjo (US, Jamshoro)	PC- 13	3. I.U. Din (USwabi)	CBGP-218
4. A.A. Babar (US, Jamshoro)	PC- 14	4. K. Mukhtar (UP, Lahore)	CBGP-219
5. S.K. Abbasi (US, Jamshoro)	PC- 15	5. M.F. Maqbool (UP, Lahore)	CBGP-220
6. A. Amur (US, Jamshoro)	PC- 16	6. T. Azeem (KUST, Kohat)	CBGP-221
7. I. Khatri (SAU, Tandojam)	PC- 17	7. A. Haseeb (KUST, Kohat)	CBGP-222
8. R. Ali (IBAU, Sakkar)	PC- 18	8. S. Abbas (UOL, Lahore)	CBGP-223
9. S. Ali (US, Jamshoro)	PC- 19	9. A. Khan (U Swabi)	CBGP-224
10. I. Khan (NIAF, Tarnab)	PC- 20	10. N. Shah (USwabi)	CBGP-225
11. N. Munawar (AAU, Rawalpindi)	PC- 21	11. A.J. Afridi (ICU, Peshawar)	CBGP-226

6:00 - 7:45 PM				
HALL-5				
SECTION III				
ENTOMOLOGY				
SESSION 4				
Chairman:		<i>Dr. Arshad Javid</i>		
Co-Chairperson:		<i>Dr. Barkat Ali Bughio</i>		
1.	F. Tahir (US, Sargodha)	ENT-51	17.	H. Ali (U Swabi) ENT-67
2.	G. Murtaza (MNSUA, Multan)	ENT-52	18.	N. Iqbal (US, Sargodha) ENT-68
3.	S. Sultan (ICU, Peshawar)	ENT-53	19.	N. Farooq (WU, AJK) ENT-69
4.	M. Shah (U Swat)	ENT-54	20.	R. Sajjad (WU, AJK) ENT-70
5.	S. Khan (U Swat)	ENT-55	21.	M. Shahbaz (WU, AJK) ENT-71
6.	S.F. Bukhari (MNSUA, Multan)	ENT-56	22.	F. Aslam (UA, Faisalabad) ENT-72
7.	S. Haleem (KUST, Kohat)	ENT-57	23.	L. Noureen (US, Jamshoro) ENT-73
8.	L.T. Kubra (US, Jamshoro)	ENT-58	24.	M. Shahbaz (MNSUA, Multan) ENT-74
9.	Z. Gul (US, Jamshoro)	ENT-59	25.	M. Kamal (UK, Karachi) ENT-75
10.	F. Siddique (US, Jamshoro)	ENT-60	26.	S. Sadiq (GCWU, Sialkot) ENT-76
11.	S.H.M. Bohari (MNSUA, Multan)	ENT-61	27.	A. Sarwar (GCWU, Sialkot) ENT-77
12.	A. Gul (MUST, Mirpur)	ENT-62	28.	F. Sard (AWKU, Mardan) ENT-78
13.	K. Saeed (MNSUA, Multan)	ENT-63	29.	M.L. Bughti (US, Jamshoro) ENT-79
14.	M. Qasim (Upponch, Rawalakot)	ENT-64	30.	M. Attaullah (U Malakand) ENT-80
15.	M.A. Rafi (Usawabi)	ENT-65	31.	S. Sanam (US, Jamshoro) ENT-81
16.	M. Younas (MNSUA, Multan)	ENT-66	32.	
8:00 PM				
DINNER				

DAY THREE**WEDNESDAY, MARCH 06, 2019****HALL-1**

9:00 AM-JOINT SESSION III: PLENARY LECTURES
CHAIRPERSON: ABDUL AZIZ KHAN
CO-CHAIRPERSON: PROF. DR. ABDULLAH G. ARIJO

1. Dr. Ijaz Ali, Department of Bio Sciences, COMSATS University, Islamabad.
"PARASITES AND HUMAN EVOLUTION"

10:00 - 11:30 AM

HALL-1			HALL-2		
SECTION I			SECTION V		
CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY			FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY		
SESSION 10			SESSION 6		
Chairman:	Dr. Bushra Muneer		Chairman:	Prof. Dr. Anila Naz Soomro	
Co-Chairperson:	Dr. Munawar Saleem Ahmad		Co-Chairperson:	Dr. Wali Khan	
1.	K. Malik (UP, Lahore)	CBGP - 17	1.	K. Irshad (GPC, Abbottabad)	FEWFM - 6
2.	S. Iqbal (UOL, Lahore)	CBGP - 18	2.	S. Parveen (UA, Faisalabad)	FEWFM - 7
3.	A. Jeelani (UVAS, Lahore)	CBGP - 19	3.	K.H. Rind (SBBU, Shaheed Benazirabad)	FEWFM - 8
4.	A. Liaqat (UP, Lahore)	CBGP - 20	4.	F.H. Shehzad (Uswabi)	FEWFM - 9
5.	I. Zahra (UP, Lahore)	CBGP - 21	5.	N.T. Narejo (US, Jamshoro)	FEWFM - 10
6.	M. Rani (MNSUA, Multan)	CBGP - 22	6.	P.Khan (US, Jamshoro)	FEWFM - 11
7.	A. Rehman (UP, Lahore)	CBGP - 23	7.	S. Jalbani (SBBU, Sakrand)	FEWFM - 12
8.	A.S. Siddiqui (UK, Karachi)	CBGP - 24	8.	M.A. Yousaf (UP, Lahore)	FEWFM - 13
9.	M.A. Saleem (UCP, Lahore)	CBGP - 25	9.	M.U. Ain (UK, Karachi)	FEWFM - 14
10.	M. Arif (UCP, Lahore)	CBGP - 26	10.	N.A. Ikram (MNSUA, Multan)	FEWFM - 15
11.	S. Yousaf (UCP, Lahore)	CBGP - 27	11.	S. Ashraf (GCU, Lahore)	FEWFM - 16
12.	A. Zafar (UCP, Lahore)	CBGP - 28	12.	K. Usman (HU Mansehra)	FEWFM - 17
13.	A. Arshad (UCP, Lahore)	CBGP - 29	13.	S. Aziz (U Swabi)	FEWFM - 18
14.	N. Riaz (UCP, Lahore)	CBGP - 30			
15.	T.A. Khichi (GPC, Gojra)	CBGP - 31			
HALL-3			HALL-4		
SECTION II			SECTION I		
PEST AND PEST CONTROL			CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY		
SESSION 7			SESSION 11		
Chairman:	Dr. Hamid Rashid		Chairman:	Dr. Raazia Tasadduq	
Co-Chairperson:	Dr. Muhammad Ishtiaq		Co-Chairperson:	Dr. Sadat Ali	
1.	T. Riaz (MNSUA, Multan)	PC - 22	1.	R. Aamir (IUB, Bahawalpur)	CBGP - 227
2.	M. Javaid (AD, Punjab)	PC - 23	2.	F. Memon (US, Jamshoro)	CBGP - 228
3.	F. Ahmad (MNSUA, Multan)	PC - 24	3.	M. Ameen (UP, Lahore)	CBGP - 229
4.	M. Ali (MNSUA, Multan)	PC - 25	4.	N. Fatimah (GCU, Lahore)	CBGP - 230
5.	W. Muhammad (AAU, R'Pindi)	PC - 26	5.	G. Jabeen (LCWU, Lahore)	CBGP - 231
6.	N. Jehanjo (US, Jamshoro)	PC - 27	6.	H. Zafar (VPCI, Karachi)	CBGP - 232
7.	S. Mushtaque (US, Jamshoro)	PC - 28	7.	M. Riaz (WU, Swabi)	CBGP - 233
8.	R.M. Shanraiz (PAD, Lahore)	PC - 29	8.	S. Aziz (GCU, Faisalabad)	CBGP - 234
9.	H.M. Tahir (GCU, Lahore)	PC - 30	9.	A. Sarwar (UA, Faisalabad)	CBGP - 235
10.	M.A. Raza (MSIA, Multan)	PC - 31			
11.	S. Abdullah (MNSUA, Multan)	PC - 32			
12.	R. Tahir (MNSUA, Multan)	PC - 33			

10:00 - 11:30 AM	
HALL-5	
SECTION III ENTOMOLOGY SESSION 4 Chairman: Dr. Alamzeb Co-Chairperson: Dr. Ayesha Aihetasham	
1. S. Hussain (U Swabi) ENT- 82 2. M. Irshad (Islamabad) ENT- 83 3. I.D. Buner (UK, Karachi) ENT- 84 4. M.S. Dayo (US, Jamshoro) ENT- 85 5. S.A. Channa (US, Jamshoro) ENT- 86 6. N. Rana (UA, Faisalabad) ENT- 87 7. T. Khan (UA, Faisalabad) ENT- 88 8. S. Saba (MNSUA, Multan) ENT- 89 9. A.M. Sabir (RRI, Kala Shah Kaku) ENT- 90 10. M. Nauman (MNSUA, Multan) ENT- 91 11. N. Khan (JUW, Karachi) ENT- 92 12. N. Majeed (UP, Lahore) ENT- 93 13. S. Akram (UP, Lahore) ENT- 94 14. A.M. Shaikh (SALU, Khairpur) ENT- 95 15. S.Zulfiqar (U Gujrat, Gujrat) ENT- 96	
11:30 AM TEA BREAK	
12:00 - 1:00 PM	
HALL-1	HALL-2
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS, TOXICOLOGY, VIROLOGY AND ANATOMY SESSION 12 Chairman: Prof. Dr. Shamsuddin A. Sheikh Co-Chairperson: Dr. Faiza Saleem	SECTION V FISHERIES, ECOLOGY AND ENVIRONMENTAL POLLUTION, FRESHWATER BIOLOGY AND FISHERIES, MARINE BIOLOGY, PALEONTOLOGY, WILDLIFE, DIVERSITY AND CONSERVATION AND BIODIVERSITY SESSION 7 Chairman: Prof. Dr. Tariq Naeem Narejo Co-Chairperson: Dr. Akhtar Rasul
1. S. Ashfaq (LCWU, Lahore) CBGP- 32 2. A. Javaid (UCP, Lahore) CBGP- 33 3. S. Batool (UCP, Lahore) CBGP- 34 4. S.Kausar (UCP, Lahore) CBGP- 35 5. H.Sadia (UCP, Lahore) CBGP- 36 6. R.Khalid (BZU, Multan) CBGP- 37 7. K.Hussain (MNSUA, Multan) CBGP- 38 8. G.M. Ghani (PH, DG Khan) CBGP- 39 9. N. Kanwal (CEMB, Karachi) CBGP- 40 10. I. Rouf (GCWU, Sialkot) CBGP- 41 11. S. Batool (QAU, Islamabad) CBGP- 42 12. U.Kinat (GCWU, Sialkot) CBGP- 43 13. B. Ali (MNSUA, Multan) CBGP- 44	1. H. Mumtaz (UA, Faisalabad) FEWFM- 19 2. A.A. Khan (UJJK, Muzafarabad) FEWFM- 20 3. S.Asif (UVAS, Lahore) FEWFM- 21 4. M.S.Khan (UOL, Sargodha) FEWFM- 22 5. I. Saleem (UOL, Lahore) FEWFM- 23 6. M.A.S.Khan (U. Swabi) FEWFM- 24 7. F.Khan (AAU, Rawalpindi) FEWFM- 25 8. M.Zaman (QAU, Islamabad) FEWFM- 26 9. N. Bano (MNSUA, Multan) FEWFM- 27 10. S.A. Shaikh (US Jamshoro) FEWFM- 28 11. S.U. Gul (KUST, Kohat) FEWFM- 29 12. M. Asad (KUST, Kohat) FEWFM- 30 13. M. Zaryab (KUST, Kohat) FEWFM- 31

12:00 - 1:00 PM			
HALL-3			
SECTION II PEST AND PEST CONTROL SESSION 8 Chairman: <i>Dr. Muhammad Ahsan Khan</i> Co-Chairperson: <i>Dr. Nuzhat Siyal</i>			
1.	P.Mari (US Jamshoro)	PC-34	
2.	M.A. Shah (US Jamshoro)	PC-35	
3.	N. Iqbal (MNSUA, Multan)	PC-36	
4.	F. Iqbal (MNSUA, Multan)	PC-37	
5.	J. Nazar (MNSUA, Multan)	PC-38	
7.	M. Iqbal (UA, Faisalabad)	PC-39	
8.	M. Arshad (LCWU, Lahore)	PC-40	
9.	W. Akram (IUB, Bahawalpur)	PC-41	
10.	M.M.Rashid (Gomal U, D.I Khan)	PC-42	
12.	F.Aslam (MNSU, Multan)	PC-43	
13.	R. Amin (MNSU, Multan)	PC-44	

1:00 - 2:00 PM	LUNCH BREAK
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CONCLUDING CEREMONY	
02:00 PM	Recitation
02:05 PM	Congress Report by President ZSP
02:35 PM	Award Ceremony
03:15 PM	Concluding Remarks by the Chief Guets
03:25 PM	Vote of Thaks

3:30 PM	REFRESHMENT
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MEMBERS OF THE CONGRESS

BAGH, AJK

Niaz, K.

BAHAWALPUR

Ameer, M.W.
Athar, R.
Ayaz, M.M. (Dr.)
Batoool, F.
Iqbal, K.
Khalil, S. (Dr.)
Khan, R.
Lashari, M. (Dr.)
Mureed, K.
Ramzan, M.
Rana, A.H.

BANU, KPK

Haseen, B.

CHITRAL

Bari, F.

FAISALABAD

Afzal, H.
Ahmad, N.
Akram, S.
Arshad, M.
Asad, M.
Aslam, F.
Fatima, Z.
Gill, N.J.
Kaukab, G.
Khalid, M.Z.
Kushf. S.
Rasheed, A.
Sadiq, H.
Sahar, S.
Ullah, I.
Zahid, H.

GUJRAT

Aftab, K. (Dr.)

ISLAMABAD

Irshad, M.

JAMSHORO

Abbasi, M.T.
Abbasi, Z.

Abro, K.K.
Abro, M.M.
Akhtar, A.
Ali, B. (Dr.)
Ali, M.
Ali, S.
Ansari, A.
Bahar, A.Z.
Bashir, M.N.
Bhanger, N.
Birmani, N.A. (Dr.)
Bozdar, M.I.
Brohi, G.H.
Bughio, S.
Chandio, A.
Channa, S.A. (Dr.)
Das, S.N. (Dr.)
Dayo, A.
Dayo, M.S.
Fatima, I.
Fatima, S.
Gul, Z.
Habib, P.
Haider, F.
Hattar, F.A.
Hingoro, S.
Jamali, R.A.
Jamil, M.
Jemi
Jokhio, J.I.
Kalhor, H. (Dr.)
Kamboh, S.B.
Khan, A.J.
Khan, P. (Dr.)
Khaskheli, S.
Khattak, N.G.
Kubra, L.
Kumar, S. (Dr.)
Kumara, V.
Lal, M.
Larik, S.A.
Mandan, A.A.
Mangi, S.
Memon, F. (Dr.)
Memon, N. (Dr.)
Memon, S.P.
Narejo, N.T. (Dr.)
Naz, S. (Dr.)
Pitafi, M.A.

Rafique, M.
Safia
Samejo, A.A.
Sanam, S.
Sanam, S.
Sanobar, N.
Shabrani, W.A.
Shaikh, F.
Shaikh, M.H.
Siyal, B.
Siyal, S.
Solangi, D.A.
Soomro, A.N. (Dr.)
Soomro, F.D.
Soomro, N.F.
Soomro, S.
Soomro, S.
Sultana, R. (Dr.)
Thebo, A.K.
Turk, J.K.
Wagan, M.S. (Dr.)

KARACHI

Buneri, I.D.
Hadi, R. (Dr.)
Iqbal, F.
Kamal, M.
Khan, A. (Dr.)
Khanam, S. (Dr.)
Khatoon, N. (Dr.)
Mewish, M.
Mustaquim, J. (Dr.)
Nisa, N. (Dr.)
Saher, N. (Dr.)
Sattar, A.
Tamimi, N.A. (Dr.)

KOHAT

Gul, I.
Rab, A.

LAHORE

Abbas, S. (Dr.)
Abdullah
Abdullah, R. (Dr.)
Abid, L.
Aftab, S.
Ahmad, Q.
Ain, Q.

Ali, M. (Dr.)
 Allah, A.
 Amjad, Q.A.
 Ara, C. (Dr.)
 Arshad, M.
 Ashfaq, H.
 Bukhari, D.A.A. (Dr.)
 Chaudhary, M.K.
 Durrani, A.Z. (Dr.)
 Faisal, M.
 Farwa
 Gardner, Q.A.A. (Dr.)
 Hamid, H.
 Hussian, M.
 Imtiaz, A.
 Imtiaz, D.
 Iqbal, A.
 Iqtedar, M. (Dr.)
 Irfan, A.
 Irshad, S. (Dr.)
 Jabeen, F.
 Javed, M.
 Jeelani, A.
 Kaleem, A. (Dr.)
 Kanwal, A.
 Khan, B.N. (Dr.)
 Khan, S.
 Khan, Z.N.
 Liaqat, I. (Dr.)
 Malik, K.
 Mehmood, M.
 Mehmood, S.
 Mirza, R.
 Mukhtar, K.
 Munir, Z.
 Naz, S. (Dr.)
 Naz, Z.
 Noreen, M.
 Perveen, S.
 Rehman, A. (Dr.)
 Rehman, S.
 Rizvi, I.H.
 Saba, S.
 Sharif, S. (Dr.)
 Shakoori, A.R. (Dr.)
 Shakoori, F.R. (Dr.)
 Siddiqua, A.
 Tabassum, R.
 Tariq, M.
 Tariq, S.
 Toor, R.H.
 Zaheer, S.
 Zahid, M.T. (Dr.)

Zaidi, S.H.A.
 Zulfiqar, S. (Dr.)

LAYYAH

Khan, A.A. (Dr.)

MALAKAND, KPK

Attaullah, M. (Dr.)
 Khan, A.R. (Dr.)
 Khan, T.

MANSEHRA

Bibi, N. (Dr.)
 Iqbal, M.
 Khan, I.
 Khan, R.A.
 Rafique, A.
 Ullah, R.
 Ullah, Z.

MARDAN

Khan, A.A.
 Said, F. (Dr.)

MIRPUR, AJK

Abbasi, A.A. (Dr.)
 Ahmed, A.
 Akram, Z.
 Alam, J.
 Ali, U.
 Aslam, S.
 Bibi, A.
 Fatima, B.
 Fazil, R.
 Gul, A.
 Islam, S.
 Javid, A.
 Khadum, F.
 Khaleeq, F.
 Khushal, S.
 Liaqat, K.
 Liaqat, T.
 Maroof, S.
 Mughal, T.A.
 Nisar, R.
 Noor, M.
 Rabnawaz, A.
 Sadia, H.
 Shafique, F.
 Shahid, T.
 Tahira, S.
 Tahseen, R.
 Ullah, R.

Waheed, H.
 Younus, K.
 Zahara, S.O.

MULTAN

Bibi, F. (Dr.)

MUZAFFARABAD

Ali, G. (Dr.)

OKARA

Wajid, M.M. (Dr.)

PESHAWAR

Ahmad, A.

QUETTA

Faheem, M.
 Kakar, J.K. (Dr.)
 Nasir, A.

RAWALPINDI

Anwar, M. (Dr.)
 Aziz, M.
 Hussain, A.
 Hussain, S. (Dr.)
 Khan, M.R.
 Mahmood, T. (Dr.)
 Muhammad, W.
 Rais, M. (Dr.)

SAKRAND, Sindh

Jalbani, S. (Dr.)

SARGODHA

Majeed, M.Z. (Dr.)

SHAHEED BENAZIRABAD (Sindh)

Rind, D.K.H. (Dr.)

SIALKOT

Sherazi, H.F.
 Zahara, S.T.

SKARDU, GILGIT – BALTISTAN

Khan, M.N. (Dr.)

SWABI

Ahmad, M.S. (Dr.)
 Khan, A.
 Khan, M.S. (Dr.)
 Rafi, M.A. (Dr.)

Rehman, H.
Shehzad, F.H.
Suleman

TANDOJAM

Ahmed, I.
Junejo, G.Q.
Khatri, I. (Dr.)

Laghari, Z.A. (Dr.)
Rajput, Z.
Siddiqui, M.W.
Soomro, R.A.

THATTA, SINDH

Mahar, M.A. (Dr.)

UTHAL, Balochistan

Bano, A. (Dr.)
Rasheed, S. (Dr.)
Ullah, M.E.

CITATIONS

RECIPIENT OF LIFE TIME ACHIEVEMENT AWARD 2019



Prof. Dr. Shamsuddin Ahmad Shaikh

Presently Chairman of Mehran Educational Society, Sindh

Prof. Dr. Shamsuddin Ahmed Shaikh fellow ZSP joined the Department, of Zoology, University of Sindh, Jamshoro as Lecturer in 1975, he earned M.Phil (1987) and Ph.D degree (1990) from Quaid-e-Azam University, Islamabad. During 37 years' of active service he involved himself in research on fish physiology and toxicology. He supervised research work of several M.Sc. and Ph.D. students. He organized Symposium in 1977, 20th Pakistan Congress of Zoology in the year 2000 and 29th Pakistan Congress of Zoology in 2009. Prof. Dr. Shaikh is chairman of Mehran Educational Society, which is a top class institution in interior Sindh.

**RECIPIENT OF
ZOOLOGIST OF THE YEAR AWARD 2019**



Dr. Nadeem Sheikh

Department of Zoology, Punjab University, Lahore

Dr. Nadeem Sheikh did his M.Sc. (Zoology) from University of the Punjab, Lahore in 1999 securing overall first class first position in the University. He was conferred Dr. Muzzafar Ahmad Gold Medal, Sir William Roberts Gold Medal and University of the Punjab Gold Medal for outstanding performance. He joined the Department of Zoology, University of the Punjab in Feb. 2001 as Lecturer in Zoology. In October 2003, he was awarded scholarship by Deutsche Forschungsgemeinschaft (DFG) for higher studies leading to Ph.D. in Georg-August University of Göttingen, Germany. His field of specialization is Cellular, Molecular and Clinical Biology of internal organs especially iron regulation during inflammation, hepatic injury, regeneration and iron overload conditions. In 2008, he was awarded Charles Wallace trust fellowship to visit Imperial College, London for one Month. Later in year 2009, he was awarded HEC postdoctoral fellowship to work in Brain Tumor Research Center of Harvard Medical School at Harvard University (USA). In 2015, Dr. Sheikh was awarded A.R. Shakoori Gold medal for his outstanding contribution in research in the area of Biological Sciences awarded by Zoological Society of Pakistan. In 2016, he was appointed Director Centre for Applied Molecular Biology (CAMB), University of the Punjab where he worked till June 2018. Dr. Sheikh is HEC recognized supervisor for Ph.D., M.S. /M. Phil research. He has authored 90 research articles in impact factor and HEC recognized journals. He has been awarded research productivity award by PCST. His h-index is 13 and i-10 index is 18. He has contributed 4 chapters in open access books. He has supervised 5 Ph.D. scholars, 41 M.S. /M. Phil, 20 B.S. / M.Sc. research thesis. He developed collaboration and linkages to various institutes nationally and internationally which lead him to bring EXPERTS (Exchange by Promoting Quality Education, Research and Training in South and South-East Asia) project for the students of the University of the Punjab and other universities of Pakistan for exchange as well as degree courses in EU Universities. His contributions for exchange and promotion of science particularly to build country's positive image abroad are appraised and acknowledged internationally.

*Other applicants of this award were Mr. Muhammad Sarwar, Faisalabad; Prof. Dr. Kausar Malik, Lahore; Prof. Dr. Muhammad Naeem Khan, Skardu; Prof. Dr. Tahira Jabeen Ursani, Jamshoro; Dr. Zafar Iqbal, Lahore; Dr. Tariq Mahmood, Rawalpindi

**RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2019**



Dr. Aamir Ali Shah

Associate Professor, Department of Microbiology, Quaid-i-Azam University, Islamabad.

He did Ph.D in 2007. Availied of Postdoctoral Fellowships by Japan Society for Promotion of Science in 2008 and Matsumaei International Foundation in 2010. His area of research interest is Applied and Environmental Microbiology. He has supervised 9 Ph.D and 58 M.Phil students in the area of Environmental Biology. His research interest involves biodegradation of plastics, purification and characterization of industrial enzymes, isolation of bioactive compounds from microbes that live in extreme environments; bio-hydrometallurgy for removal of metals from soil and water by leaching and sorption techniques. Presently Dr. Shah is supervising 08 Ph.D and 07 M.Phil students. He has published 96 research articles with cumulative impact factor of 178, citations 2230 and h-index 15. Pakistan Academy of Sciences awarded him “Gold Medal in Biological Sciences for the year 2018”. He also several Research Productivity Award from the Pakistan Council for Science & Technology in the year 2010, 2011, 2012 and 2014. Dr. Shah has published a book on title “Role of microorganisms in biodegradation of plastics in 2009.

*Other applicants of this award were Dr. Iram Liaqat, Lahore; Dr. Muhammad Imran Qadir, Multan; Dr. Hafiz Muhammad Tahir, Lahore; Dr. Saima Naz, Jamshoro; Dr. Samreen Riaz, Lahore; Dr. Muhammad Sajjad Ansari, Sargodha; Dr. Zulfiqar Ali Mirani, Karachi; Dr. Furhan Iqbal, Multan; Dr. Nisar Ahmad, Swat; Dr. Azhar Rasul, Faisalabad; Dr. Muhammad Khan, Lahore; Dr. Muhammad Ijaz, Lahore; Dr. Muhammad Ali, Islamabad; Dr. Faiza Saleem, Lahore; Dr. Riffat Sultana, Jamshoro.

**RECIPIENT OF
PROF. IMTIAZ AHMAD GOLD MEDAL 2019**



Dr. Muhammad Zeeshan Majeed

Department of Entomology, College of Agriculture, University of Sargodha, Sargodha.

Dr. Muhammad Zeeshan Majeed earned has B.Sc. (Hons.) Agriculture in 2003 and M.Sc. (Hons.) Agriculture degrees with Entomology as specialization in 2005 from the University of Agriculture, Faisalabad. He joined University of Sargodha as Lecturer in the Department of Entomology, College of Agriculture. Later he obtained his 2nd Master degree in Microbiology and Parasitology in June 2009 and Doctorate degree in Insect Microbial Ecology in December 2012 from the Université de Montpellier-II. He got Best Research Paper Award from HEC in 2014. He won Talented Young Scientist Postdoctoral Fellowship Award by the Ministry of Science & Technology, China and completed his postdoc research in 2017-2018 from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China.

*Other applicant of this award was Dr. Muhammad Babar Shahzad Afzal, Sargodha

**RECIPIENT OF
DR. ABDUL ALEEM CHAUDHARY GOLD MEDAL 2019**



Dr. Muhammad Rais

Assistant Professor, Department of Wildlife Management, Arid Agriculture University, Rawalpindi.

He obtained his M.Sc. degree in 2004 and Ph.D. degree in 2013 from University of Karachi. He joined PMAS-Arid Agriculture University Rawalpindi in 2007 as a lecturer in Wildlife Management, and was later appointed as an Assistant Professor in 2015. He has supervised 21 MPhil and 5 MSc students while 5 PhD and 6 MPhil scholars are currently enrolled with him. He has established linkages with herpetologists in Deakin University Melbourne and University of Canberra, Australia; Purdue University West Lafayette, USA and researchers in India, Sri Lanka, South America and Europe. He has obtained training in herpetology in India in 2012, in movement patterns in reptiles in USA in 2012-2013. Australian Government's prestigious six months training in thermal biology, captive breeding and use of radio-telemetry on endemic lizards in Australia under the sponsorship of Endeavor Post-doc scholarship in 2014-2015. He has published over 40 research articles in reputable journals. He has completed an international research project worth US \$ 12,000, funded by International Foundation for Science, Sweden on amphibian ecology and conservation. He is currently conducting research project (Rs.8.95 million) dealing with taxonomy, distribution, occupancy modeling, home range, population monitoring and impact of land use, land cover change and climate change on amphibians and reptiles.

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

1. Muzaffer Ahmad Gold Medal 2019

The 26th Muzaffer Ahmad Gold Medal 2019 was received by Ayesha Ms. Ayub a student of the University of the Punjab, Lahore for standing first in the recent M.Sc. Zoology examination.



Ms. Ayesha Ayub

2. Afsar Mian Gold Medal 2019

Eleventh Afsar Mian Gold Medal 2019 was given to Ms. Hafsa Yaseen a student of the Arid Agriculture University, Rawalpindi for standing first in the recent M.Sc. Biology/Zoology examination.



Ms. Hafsa Yaseen

3. Muhammad Afzal Hussain Qadri Memorial Gold Medal 2019

The 22nd Muhammad Afzal Hussain Qadri Memorial Gold Medal 2019 was awarded to Ms. Ismat Arshad student of Karachi University for standing first in the recent M.Sc. Zoology examination.



Ms. Ismat Arshad

4. Prof. Dr. S.N.H. Naqvi Gold Medal 2019

The 15th Prof. Dr. S.N.H. Naqvi Gold Medal 2019 was given to Dr. Zubair Ahmed for obtaining Ph.D. degree in Zoology with specialization in the field of Toxicology from University of Karachi.

5. M.A.H. Qadri Memorial Gold Medal 2019

The 19th M.A.H. Qadri Memorial Gold Medal 2019 was given to Dr. Farah Sharif for obtaining Ph.D. degree in Zoology with specialization in the field of Parasitology from University of Karachi.

6. Mujib Memorial Gold Medal 2019

The 26th Mujib Memorial Gold Medal 2019 was given to Ms. Ismat Arshad a student of Karachi University for standing first in the recent M.Sc. Zoology examination with specialization in Parasitology.

7. Prof. Dr. Muhammad Ali Gold Medal 2019

The 3rd Prof. Dr. Muhammad Ali Gold Medal 2019 was awarded to Ms. Komal Mahmood a student of Government College University, Faisalabad for standing first in the recent M.Sc. Zoology examination.



Ms. Komal Mahmood

8. Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2019

The 3rd Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2019 was awarded to Mr. Musavir Ali Channar a student of University of Sindh, Jamshoro for standing first in the recent final B.S. Examination of Freshwater Biology & Fisheries.



Mr. Musavir Ali Channar

9. Ahmed Mohiuddin Memorial Gold Medal 2019

The 14th Ahmed Mohiuddin Memorial Gold Medal 2019 was awarded to Ms. Ameerza a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination.

10. Prof. Dr. S.S. Akbar Memorial Gold Medal 2019

The 6th Prof. Dr. S.S. Akbar Memorial Gold Medal 2019 was awarded to Ms. Anum Fatima a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination with specialization in Entomology.

11. Prof. Dr. Muhammad Saeed Wagon Gold Medal 2019

The 1st Prof. Dr. Muhammad Saeed Wagon Gold Medal 2019 was awarded to Ms. Fatima Anum a student of University of Sindh, Jamshoro for standing first in the recent BS-IV Zoology examination.



Ms. Fatima Anum

AWARDED SHIELD AND CERTIFICATE
for his contributions towards promotion of Zoological Society
in his capacity as Life-Fellow of ZSP.

Prof. Dr. Malik Muhammad Anwar

Department of Zoology, Government College University, Lahore

AWARDED SHIELD AND CERTIFICATE
for Late Prof. Dr. Muhammad Nasim Siddiqui in recognition of
his contributions in the field of General Zoology

Late Prof. Dr. Muhammad Nasim Siddiqui

Prof. Dr. Syed Akram Shah, Chairman, Department of Zoology, University of Peshawar received a Posthumous Shield and a Certificate for Late Prof. Dr. Muhammad Nasim Siddiqui

Some Glimpses of Academic Sessions and the Congress Participants



Section of audience at the Inauguration Day of PCZ



Section of audience during inaugural session



Prof. Dr. A.R. Shakoori, President, Zoological Society of Pakistan addressing at the inaugural session.
Dr. Ali Muhammad Yousaf Zai and Dr. Abdul Rehman at the back



Three Senior Zoologists (Left to Right)
Mr. A.A. Khan, Prof. Dr. Muhammad Anwar, Prof. Dr. A.R. Shakoori



Prof. Dr. Shamsuddin Sheikh receiving Life Time Achievement Award from the Vice Chancellor, Prof. Dr. Habib Ahmad. The President and the Secretary ZSP are also present



Prof. Dr. Habib Ahmad, Vice Chancellor, Islamia College University Peshawar giving medal & shield to a medal winner. Dr. A.R. Shakoory and Mr. A.A. Khan accompanying the VC at stage



Prof. Dr. A.R. Shakoori giving shield of appreciation to Mr. A.A. Khan



Some of the participants and members of organizing committee of the Congress
Syed Haider Ali Zaidi, (2nd from left), Mr. Qadeer Ahmad (4th from left)
and Mr. Javed Ahmed (1st from left) are prominently visible



Biodiversity of Invertebrates in some Selected Areas of District Bagh, Azad Jammu and Kashmir, Pakistan

Khifza Niaz, Abu Ul Hassan Faiz*, Azaz Ahmad and Muhammad Shabaz

Women University of Azad Jammu and Kashmir, Bagh, Azad Jammu and Kashmir, Pakistan

ABSTRACT

The present study was designed to find species composition, diversity of insects existing at different trophic level of food chain in coniferous forest of District Bagh. The study was conducted from September, 2017 to September, 2018. The researcher recorded 61 species of insects belonging to 51 families. Among the identified species of invertebrates, 32 species are herbivore, 19 species are carnivore (insect predator), 8 species of omnivores and 2 species are scavengers. The herbivore insect species feed on 134 plants spp. of the study area. The greater population density of herbivore indicate that plants are under more threat to insect pests. The present study provides base line information about the insect community of forest and need further screening of insect pest species to control pest out break for conservation of coniferous forest District Bagh.

Article Information

The article was presented in 39th Pakistan Congress of Zoology (International) held on 4-6th March 2019, organized by Islamia College University, Peshawar, Pakistan.

Authors' Contribution

KN conducted the field work. AHF wrote the manuscript. AA and MS performed taxonomy of specimen.

Key words

Trophic level, Insect predator, Carnivore, Herbivore

INTRODUCTION

Natural ecosystems provide a variety of services, such as provisioning services (production of food, fiber, water), cultural services (recreation, spiritual and aesthetic), supporting services (pollination, decomposition and soil formation) and regulating services (biological control), while disservices of ecosystems are such as pests, litter, diseases, animal attacks, allergenic and poisonous organisms (Zhang *et al.*, 2007). The disservices of ecosystem are exacerbated by anthropogenic destabilization of food webs, ecosystem structures and appear in the form of floods, storms and weather events (Ratcliffe *et al.*, 2011). The insects ensure sustainability of ecosystem services and minimize induction of disservices such as famine, water shortages, threats to human health and economic disruption (Lentz and Hockaday, 2009). The unsustainable exploitation of ecosystem services produces risks of human health in the form of epidemics of crowd diseases, often vectored by insects (Brouqui *et al.*, 2011). Insects deserve particular attention because they play dual role in ecosystem services and disservices of significant economic value (Wegier *et al.*, 2017). A lot of studies on Lesser Himalayas have been done by Faiz *et al.* (2014) and Faiz and Fakhr (2015a, 2015b, 2015c). The present study has been organized to provide a perspective on checklist of insects as predator, herbivore and carnivore.

MATERIALS AND METHODS

The study was carried out in cultivated, suburban and wild areas of district Bagh, Azad Jammu and Kashmir from November 2017 to September 2018 (during day from 09:00 am to 02:00 pm). Bagh is a mountainous area from northeast to south-west with elevation is between 1500 and 2500 meters asl, lies in the lesser Himalayas zone of Pir-Panjal. The main eastern part of the district is very cold in winter and moderate in summer. The specimens were collected from wild areas, cultivated and suburban areas of Bagh, AJK during their active season by light trap, pit fall, sweep net and hand-picking method by taking 12 transects on each study site. The collected specimens were killed in killing bottle containing formalin. The specimens were then kept in insect boxes. The specimens were identified with the help of available literature.

RESULTS AND DISCUSSION

A total of 60 species belonging to 50 families were recorded in study area. In family Attelabidae, the herbivores species, leaf rolling weevil (*Apoderus* sp.) was collected from sub-urban areas, cultivated areas and wild study sites. Geographic distribution of this species is worldwide. In family Acrididae, one herbivore species, short horned grasshopper (*Chorthippus*) was collected from sub-urban areas and cultivated areas. The geographic distribution of this species was worldwide. In family Apidae, two herbivores species, giant honey bee (*A. dorsata*) and

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0030-9923/2019/0039-0001 \$ 9.00/0
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Table I. Check list of invertebrates observed in study area.

Family	Scientific name	Local name	Sub-urban	Cultivated	Wild
weevil					
Attelabidae	<i>Apoderus</i> sp.	Leaf rolling weevil	✓	✓	✓
Grasshopper					
Acrididae	<i>Chorthippus</i> sp.	Short horned grasshopper	✓	✓	✓
Bees					
Apidae	<i>A. dorsata</i>	Giant honey bee	✓	✓	✓
	<i>A. mellifera</i>	European honey bee	✓	x	✓
Beetles					
Buprestidae	<i>Sternocera</i> sp.	Jewel beetle / Metallic wood-boring beetle		x	
Cerambycidae	<i>B. rufomaculata</i>	Mango stem borer beetles	✓	✓	✓
	<i>A. serraticornis</i>	Long horn beetle	✓	✓	✓
Chrysomelidae	<i>Raphidopalpa</i> sp.	Leaf beetle	✓	✓	✓
Scarabaeidae	<i>Phyllophaga</i> sp.	June beetle	✓	✓	✓
Carabidae	<i>C. auratus</i>	Ground beetle	✓	✓	✓
Coccinellidae	<i>C. septempunctata</i>	Ladybird beetle	✓	✓	✓
Elateridae	<i>Pyrophorus</i> sp.	Click beetle	✓	✓	✓
Oedemeridae	<i>Ananca</i> sp.	Red-black oedemerid	✓	✓	✓
Lucanidae	<i>Lucanus</i> sp.	Stage beetle	✓	✓	✓
Scarabaeidae	<i>Coprisrepertus</i> sp.	Dung beetle	✓	✓	✓
Tenebrionidae	<i>Stenochinu</i> sp.	Darkling beetle	✓	✓	✓
Wasps					
Braconidae	<i>Microgastrinae</i> sp.	Braconid wasp	✓	✓	✓
Moth					
Erebidae	<i>E. confinis</i>	Wasp moth	✓	✓	✓
	<i>Spilosoma</i> sp.	Tiger moth	✓	✓	✓
Geometridae	<i>Rhodometra</i> sp.	Geometer moth	✓	✓	✓
	<i>Scopula</i> sp.	Emerald moth	✓	✓	✓
Crickets					
Gryllotalpidae	<i>Gryllotalpa</i> sp.	Mole cricket	✓	✓	✓
Gryllidae	<i>Gryllus</i> sp.	Cricket	✓	✓	✓
Tettigoniidae	<i>Amblycorypha</i> sp.	bush cricket	✓	✓	✓
Bugs					
Lygaeidae	<i>Spilostethus</i> sp.	Lygaeidae bug	✓	✓	✓
Largidae	<i>Physopelta</i> sp.	Largidae bug	✓	✓	✓
Tessaratomidae	<i>T. javanica</i>	Giant shield bug	✓	✓	✓
Oniscidae	<i>Oniscus</i> sp.	Sow bug	✓	✓	✓
Nabidae	<i>N. ferus</i>	Damsel bug	✓	✓	✓

Continued on next page.....

Family	Scientific name	Local name	Sub-urban	Cultivated	Wild
Reduviidae	<i>Rhynocoris</i> sp.	Nymph of assassin bug	✓	✓	✓
Cicadidae	<i>Cicada</i> sp.	true bugs	✓	✓	✓
Anisolabididae	<i>Euborellia</i> sp.	Earwig bugs	✓	✓	✓
Butterflies					
Nymphalidae	<i>D. chrysippus</i>	Plain tiger (Butterfly)	✓	✓	✓
	<i>A. hyperbius</i>	Tropical or Indian fritillary	✓	✓	✓
Pieridae	<i>P. brassicae</i>	Cabbage butterfly	✓	✓	✓
Papilionidae	<i>P. bianor</i>	Common peacock	✓	✓	✓
Glow-Worm					
Keroplidae	<i>A. luminosa</i>	Glow worm	✓	✓	✓
Noctuidae	<i>A. Ipsilon</i>	Cutworm	✓	✓	✓
Scorpions					
Buthidae	<i>Hottentotta</i>	Indian red scorpion	✓	✓	✓
	<i>Scorpiops</i>	Fattail scorpion	✓	✓	✓
	<i>Chaerilus celebensis</i>	speckled bush scorpion	✓	✓	✓
Mantidae	<i>M. religiosa</i>	European mantis	✓	✓	✓
Amorphoscelidae	<i>H. ceylonica</i>	Bark mantis	✓	✓	✓
Spiders					
Erebidae	<i>Lycosa</i> sp.	Wolf spider	✓	✓	✓
Hersiliidae	<i>Hersilia</i> sp.	Two tailed spider	✓	✓	✓
Lycosidae	<i>Alopecosa</i> sp.	Wolf spider	x	✓	✓
Thomisidae	<i>Pistius</i> sp.	Crab spider	✓	✓	✓
Salticidae	<i>Plexippus</i> sp.	Jumping spider	✓	✓	✓
	<i>T. dimidiata</i>	Araneomorph spider	✓	✓	✓
Dragonfly species					
Libellulidae	<i>O. glaucum</i>	Blue marsh hawk	x	✓	✓
	<i>O. glaucum</i>	Red marsh hawk	✓	✓	✓
Ants					
Formicidae	<i>C. compressus</i>	Carpenter ant	✓	✓	✓
	<i>Dorylinae</i> sp.	Male doryline ant	✓	✓	✓
	<i>L. niger</i>	Alate ant	x	✓	✓

European honey bee (*A. mellifera*) were collected from all study sites. In family Buprestidae, species jewel / metallic wood-boring beetle (*Sternocera*) was collected from cultivated areas and wild study site. In family Cerambycidae, two species (*B. rufomaculata* and *A. serraticornis*) were recorded. Both species were present at sub-urban and wild area and cultivated area. The geographic distribution of these species is worldwide. In family Chrysomelidae, only one species (*Raphidopal* sp.) was recorded. This species was present at all three

study sites (cultivated, sub-urban and wild area). In family Scarabaeidae, only one species june beetle (*Phyllophaga* sp.) was recorded in all study sites. In family Carabidae, only one species ground beetle (*C. auratus*) was recorded at all three study sites. In family Coccinellidae, only one species ladybird beetle (*C. septempunctata*) was recorded. at one study site. In family Elateridae, only one species click beetle (*Pyrophorus* sp.) was recorded at all three study sites. In family Oedemeridae, only one species (*Ananca*) was recorded at all three study sites. In family

Lucanidae, only one species stag beetle (*Lucanus* sp.) was recorded at all study sites. In family Scarabaeidae, only one dung beetle (*Coprisrepertus* sp.) was recorded and present at all three study sites. In family Tenebrionidae only one darkling beetle (*Stenochinus* sp.) was recorded at all three study sites. In family Braconidae, only one barconid wasp (*Microgastrinae* sp.) was recorded at all three study sites. In family Erebididae, only one species wolf spider (*Lycosa* sp.) was recorded at all three study sites in family Geometridae, two species (*Rhodometra* and *Scopul*) were present at all the three study sites. In family Gryllotalpidae, only one species of mole cricket (*Gryllotalpa* sp.) was present at all three study sites. In family Tettigoniidae, only one species leaf mimicking katydid (*Amblycorypha* sp.) was recorded at all the three study sites.

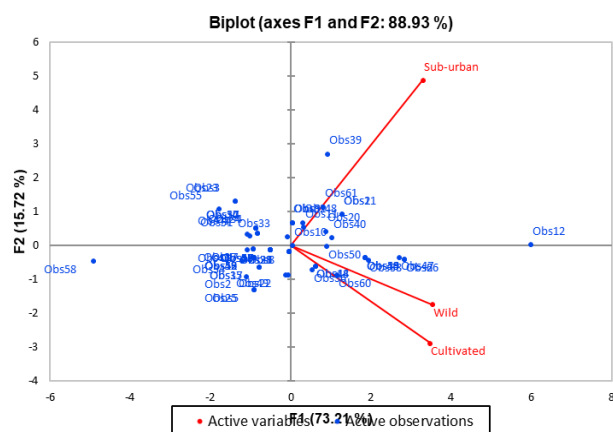


Fig. 1. Distribution of species in study area.

In family, Lygaeidae, only one species, Lygaeidae bug (*Spilostethus* sp.) was recorded. This species was present at all study sites. In family Largidae, only one (*Physopelta* sp.) was recorded. This species was present at all three study sites. In family Tessaratomidae, only one species nymph of giant shield bug (*T. javanica*) was recorded at all study sites. In family, Oniscidae only one species (*Oniscus*) was recorded at cultivated, sub-urban area and wild area In family Nabidae, only one species Damsel bug (*N. ferus*) was recorded and present at all study sites. In family Reduviidae, only one species, nymph of assassin bug (*Rhynocoris* sp.) was recorded at all three study sites. In family Cicadidae, only one herbivore species (*Cicada*) was recorded at sub-urban, wild area cultivated area. In family Nymphalidae, two species (*D. chrysippus* and *A. hyperbius*) were recorded at all three study sites. In family Pieridae, only one species cabbage butterfly (*P. brassicae*) was recorded at all three study

sites. In family Papilionidae, only one species common peacock (*P. bianor*) was recorded at all three study sites. In family Keroplatidae, only one species glow worm (*A. luminosa*) was recorded at all study. In family Noctuidae, only one species cut worm (*Noctuapronuba*) was recorded at cultivated, wild area and in sub-urban area. In family Buthidae, only one species scorpion (*Hottentotta* sp.) was recorded at all three study sites. In family Salticidae, two species (*Plexippus* sp. and *T. dimidiata*) were recorded at all three study sites (cultivated, suburban and wild). In family Amorphoscelidae, only one species, bark mantis (*H. ceylonica*) was recorded at all two study sites and was absent in wild area.

In family Erebididae, two species (*E. confinis*) and (*Spilosoma* sp.) were recorded. Both species were present at all three study sites. In family Hersiliidae, only one species, two tailed spider (*Hersilia* sp.) was recorded at all study sites. In family Lycosidae, only one (*Alopecosa* sp.) was recorded at all three study sites. In family Thomisidae, only one species crab spider (*Pistius* sp.) was recorded at all three study sites (cultivated, sub-urban and wild area. In family Libellulidae two species blue marsh hawk and red marsh hawk was recorded at all three study sites. In family Formicidae three species were recorded, *C. compressu*, *Dorylinae* sp. and *Lasiusniger* which were present at all three study sites. In family Anisolabididae, the herbivore species earwig (*Euborellia* sp.) was collected from sub-urban areas, and wild study sites. In family Erebididae, two species (*E. confinis* and *Spilosoma* sp.) were recorded. Both species were present at all three study sites.

Statement of conflict of interest

The authors declare there is no conflict of interest.

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Genotoxic Potential of Pesticide Mixture against Common Carp

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ABSTRACT

Contamination of water due to pesticide residues is a global concern. Pesticides come from industrial and agricultural effluents, reach in the aquatic environment where it poses significant toxicological risks to non target organisms. Pesticides find their way to the food chain threatening the ecological balance and biodiversity of the natural ecosystems. Therefore, this study was designed to determine the genotoxic potential of two commonly used pesticides viz. chlorpyrifos and bifenthrin in the form of mixture on commercially important freshwater fish common carp (*Cyprinus carpio*). For this purpose, 180-day old fish fingerlings were exposed to sub-lethal concentrations viz. 1/3rd, 1/4th, 1/5th and 1/6th of 96-hr LC₅₀ of mixture along with negative and positive control for a period of two months at constant laboratory conditions. Fish blood erythrocytes were sampled on day 30th and 60th of exposure period for the assessment of DNA damage. Genotoxic effects of mixture were determined in terms of percentage of damaged nuclei in the peripheral blood erythrocytes of fish. Statistically significant effects ($p < 0.05$) for both concentration and time of exposure were observed in treated and control fish. The level of DNA damage in terms of percentage of damaged nuclei was significantly higher on day 60th of exposure at all sub-lethal concentrations as compared to 30th day. The DNA damage was found to be dose and time dependent with highest DNA damage observed at 1/3rd of LC₅₀, followed by that of positive control, 1/4th, 1/5th and 1/6th of LC₅₀ exposures as compared to negative control. This study confirmed that the comet assay is a useful tool for assessing the genotoxic potential of waterborne pollutants and might be appropriate as a part of environmental monitoring programs.

Article Information

The article was presented in 39th Pakistan Congress of Zoology (International) held on 4-6th March 2019, organized by Islamia College University, Peshawar, Pakistan.

Authors' Contribution

FA performed experiments and wrote this article. SK statistically analyzed the data. RI and SA helped in the write-up of the manuscript. MJ reviewed the final manuscript.

Key words

Genotoxicity, DNA damage, Sublethal, Pesticide

INTRODUCTION

Pesticides are now heavily used in agricultural fields to control the pest population. These pesticides are usually very toxic to non target organisms like fish and play important role in the global loss of freshwater fish biodiversity (Ramussen *et al.*, 2015). Pesticides comprise an extensive range of synthetic organic compounds (Lazartigues *et al.*, 2013) and these compounds are among more than 1000 active ingredients that are marketed as herbicides, insecticides and fungicides (Mostafalou and Abdollahi, 2013). As a well known organophosphate pesticide, chlorpyrifos is extensively used for controlling agriculture and house hold pests all over the world (Yen *et al.*, 2011). In 2007, chlorpyrifos was the 14th most commonly used conventional active ingredient in the

agricultural pesticide market sector (Grube *et al.*, 2011). Soil erosion, runoff events along with leaching are the major routes of chlorpyrifos entry into the surface water (Jin *et al.*, 2015). While another class of pesticides; pyrethroid is also extensively used, and some evidence indicates that these chemicals are gonotoxic for many organisms including fish. Bifenthrin is active insecticide belongs to pyrethroid group of pesticide and is used in agriculture and public health control programs especially for the control of mosquitoes. In fish, it may affect the central and peripheral nervous system and cause synaptic discharge, depolarization and ultimately cause death (Ponepal *et al.*, 2010).

Exposure to pesticides in animal's sometime result in heritable damage or inactivation of DNA, such phenomenon is called genotoxicity. The genotoxic potential of various pesticides may be attributed to its potential to act on genetic information and altering the structure of DNA, so interfere with process of transcription, replication and translation (Dogan *et al.*, 2011). Geno-toxicants like pesticides have

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0030-9923/2019/0039-0007 \$ 9.00/0
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similar chemical and physical properties that enable them to interact with genetic material. Therefore, there is a growing concern over the existence of these genotoxicants in the aquatic environment because they pose severe negative impact on fish health. Presence of genotoxicants in the aquatic ecosystem is a well known reality, several attempts to develop a sensitive biomarkers to evaluate the genotoxic effects in aquatic fauna has gained the importance from last few decades. Among different techniques, the single cell gel electrophoresis (SCGE) also known as comet assay is recognized as one of the most reliable and sensitive technique available for DNA strand break detection with the advantage of being simple, sensitive and rapid (Mitchellmore and Chipman, 1998). Contaminants usually present as a complex mixtures in natural water. The cocktails of compounds create severe problem as toxicity of a mixture is not easily linked to individual toxicities of components in the mixture. Studies associated with complex mixtures geno-toxicity are scarce, especially considering the environment realistic mixture concentrations.

The common carp (*Cyprinus carpio*) was selected as the test organism because it is among the most economically important freshwater fishes of the world. There is a scarcity of information regarding the genotoxicity of pesticide mixture on freshwater fishes therefore; the aim of the present study was the detection of possible dose dependent in-vivo genotoxicity of binary pesticides mixture (chlorpyrifos+bifenthrin) in *Cyprinus carpio* during 60-day trial.

MATERIALS AND METHODS

The *Cyprinus carpio* (180-day old) were purchased from local fish seed hatchery and transported to Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. Healthy fingerlings of similar weight and length were acclimatized under laboratory conditions in cemented tanks for about two weeks and fed with nutritious diet. Chlorpyrifos and bifenthrin were dissolved, separately, in 95% analytical grade methanol (J.T. Baker) as a carrier solvent to prepare the stock-I solutions (1g/100 ml) while binary mixture of these pesticides were prepared by its further dilutions in deionized water (stock-II). The acute toxicity bioassay to determine the 96-hr LC_{50} value of pesticide mixture (chlorpyrifos+bifenthrin) was conducted in static system. The 96-hr LC_{50} was determined as $0.76 \mu\text{g L}^{-1}$ for *Cyprinus carpio* by using the Probit analyses method. Based on this 96-hr LC_{50} value, test concentrations i.e. $1/3^{\text{rd}}$, $1/4^{\text{th}}$, $1/5^{\text{th}}$ and $1/6^{\text{th}}$ of LC_{50} values were calculated and used for the assessment of sub-lethal genotoxicity experiment.

Single cell gel electrophoresis (SCGE)

The experiments were conducted in glass aquaria to determine the extent of DNA damage in the peripheral blood erythrocytes of *Cyprinus carpio*. For this purpose, the fifty fingerlings of *Cyprinus carpio* were divided into six groups. First group were exposed to $1/3^{\text{rd}}$ of pesticide mixture (test concentration), second group to $1/4^{\text{th}}$, third to $1/5^{\text{th}}$, fourth to $1/6^{\text{th}}$, fifth group were maintained in tap water considered as “Negative Control” (unstressed group), while in sixth group cyclophosphamide ($20 \mu\text{g g}^{-1}$) was used as “Positive Control”. During whole experimental period fish were fed daily with small quantity of food. Water temperature (30°C), pH (7.75) and total hardness (225 mg L^{-1}) were kept constant throughout the experimental duration. Exposure was continued for 60 days and blood erythrocytes slides were prepared on monthly basis from all experimental groups and subjected to alkaline single cell gel electrophoresis (Comet assay). Experiment was conducted with three replications. Alkaline single cell gel electrophoresis was performed as three layer procedure by following the methods of Singh *et al.* (1988). Blood samples were collected from caudal vein of fish transferred in eppendorf and treated with anticoagulants and diluted with 1 ml of phosphate buffer saline (PBS). Blood ($60 \mu\text{l}$) was mixed with $110 \mu\text{l}$ of 1.7% low melting point (LMP) agarose. Sample mixture then layered on the glass slides pre-coated with 0.5% normal melting point (NMP) agarose and immediately covered with a cover slip and kept for 10 minutes in a refrigerator to solidify. After gently removing the cover slips, the slides were coated with a third layer of $75 \mu\text{l}$ low-melting point agarose and covered with glass slip again. After solidification of gel the slides were immersed in cold lysing solution and refrigerated at 4°C , followed by electrophoresis (25 V, 300 mA for 25 minutes) and staining with ethidium bromide. Two slides were prepared and one hundred and fifty cells per slide were scored randomly and analyzed by using an image analysis system attached to Epi-Fluorescence microscope (N-400M, American Scope; UK) equipped with light source of mercury short arc reflector lamp filters for ethidium bromide at 400 X magnification and low lux (MD-800, American Scope; UK) camera.

The DNA damage was quantified by visual classification of cells into four classes of “comets” corresponding to the tail length (measured through Tri Tek Comet Score™) as undamaged (Type-0); low level damage (Type-I); medium level damage (Type-II); high level damage (Type-III) and complete damage (Type-IV). All steps were conducted in dim light to avoid any non-specific additional breakage of DNA. The extent of DNA damage was examined as the mean percentage of cells with medium, high and complete damaged DNA, which was

calculated as the sum of cells with class II+III+IV. Statistical analyses were performed by using the MSTATC computer software. Results were expressed as Means \pm SD. A p-value less than 0.05 were considered statistically significant. Means of data were compared for the statistical differences by using Duncan Range Multiple tests (Steel *et al.*, 1996).

RESULTS

The genotoxic effect of chlorpyrifos and bifenthrin mixture was investigated by using the single cell gel electrophoresis or comet assay. Table I shows significantly variable proportions of undamaged (normal cells) and damaged nuclei (Type-I to Type-IV) in the peripheral blood erythrocytes of *Cyprinus carpio* under exposure of 1/3rd, 1/4th, 1/5th and 1/6th of LC₅₀ along with negative and positive control during 60-day trial.

Day 30th of exposure period

It was observed that proportion of damaged cells changed with the various concentrations (1/3rd, 1/4th, 1/5th, 1/6th, negative control and positive control) of pesticides mixture (chlorpyrifos+bifenthrin). *Cyprinus carpio* showed significantly higher percentage of Type-0 as evident by their mean value of 98.00 \pm 0.00 due to negative control treatment, followed by that of positive control, 1/5th, 1/4th, 1/6th and 1/3rd of LC₅₀. However, non significant differences were observed between 1/4th and 1/6th of LC₅₀ exposure regarding induction of Type-0 or undamaged nuclei. Statistically significant (p<0.05) maximum and minimum Type-I nuclei (low level damage) were observed due to 1/6th of LC₅₀ and negative control treatment, respectively. Exposure of pesticides mixture induced significantly higher percentage of Type-II and Type-III damaged nuclei due to 1/4th and 1/3rd LC₅₀ exposure, respectively. In the present study, DNA damage showed dose dependent relationship. The percentage of Type-IV damaged nuclei were observed higher under positive control and 1/3rd of LC₅₀ exposure while same was lower under negative control treatment. The extent of DNA damage was examined as the mean percentage of nuclei with medium, high and complete DNA damage, which was calculated as the sum of Type-II, III and IV. The DNA damage determined in terms of percentage of damaged nuclei the fish peripheral blood erythrocytes varied significantly due to exposure of chlorpyrifos+bifenthrin mixture to selected fish. Highest mean percentage of damaged nuclei with value of 64.00 \pm 0.00 was observed at 1/3rd of LC₅₀ exposure as compared to negative control.

Day 60th of exposure period

Among all test treatments, the proportion of Type-0 or

undamaged nuclei were observed higher in control group. However, percentage of Type-I damaged nuclei were maximum (36.67 \pm 3.06%) due to 1/6th of LC₅₀ exposure as compared to control treatments. The percentage of Type-II damaged nuclei were ranged from 0.00 \pm 0.00 to 34.00 \pm 3.46% and exhibited concomitant increase in DNA damage with increase in exposure concentrations of 1/6th to 1/3rd of LC₅₀ as compared to control. The exposure of fish to 1/3rd of LC₅₀ caused significantly (p<0.05) higher percentage of Type-III nuclei as compared to negative and positive control. However, the percentage of Type-IV nuclei (completely damaged nuclei) were observed higher under positive control treatment, closely followed by 1/3rd of LC₅₀ exposure while same was lower under negative control. Statistically significant (p<0.05) DNA damage was observed at 60th day of exposure due to different test concentrations/treatments. Regarding different sublethal treatments (1/3rd, 1/4th, 1/5th, 1/6th LC₅₀, negative and positive control), the extent of DNA damage was observed significantly higher due to 1/3rd of LC₅₀ exposure, followed by that of 1/4th, positive control, 1/5th, 1/6th and negative control indicating dose dependent DNA damage.

DISCUSSION

Increasing concern for the environmental genotoxicity studies has led to the development of various tests and techniques for the detection of genotoxic substances in the aquatic ecosystem. The present study endeavors the genotoxic potential of binary pesticides mixture in *Cyprinus carpio* by using comet assay. Selected fish were exposed (for 60 days) to sublethal concentrations of pesticides mixture viz. 1/3rd, 1/4th, 1/5th and 1/6th of LC₅₀ and compared with control (negative and positive control). In the present study, the tested sublethal concentrations could be environmentally relevant concentrations. Pesticides in sublethal concentration present in water are too low to cause sudden death but may affect the normal functioning of the organisms, impair their behavior, affect their growth and development and ultimately reduce the survival rate of population (Susan *et al.*, 2010).

The long term genotoxicity studies can be an important approach for achieving the greater insight into DNA repair ability of organism and for other defensive mechanisms for excreting the xenobiotics. Presort experiment showed that exposure of fish to various sublethal concentrations of pesticide mixture induced significantly higher DNA damage in blood cells of fish as compared to control. To study the relationship between DNA damage of aquatic organisms and pollutants, the comet assay is widely used for the determination of genotoxic potential of environmental pollutants in organisms (Lee and Steinert, 2003).

Table I. DNA damage in peripheral erythrocytes of *Cyprinus carpio* exposed to chlorpyrifos+bifenthrin mixture exposure.

	Undamaged nuclei (%)	Damaged nuclei (%)					Percentage of damaged nuclei (II+III+IV)
Exposure Treatments duration	Type-0	Type-I	Type-II	Type-III	Type-IV		
Day 30 th of exposure							
1/3 rd of LC ₅₀	14.67±1.15 e	21.33±1.15 d	17.33±3.06 bc	26.67±1.15 a	20.00±2.00 a	64.00±0.00 a	
1/4 th of LC ₅₀	19.33±1.15 d	36.67±3.06 c	20.00±2.00 ab	12.67±3.06 cd	11.33±1.15 b	44.00±2.00 c	
1/5 th of LC ₅₀	26.00±3.46 c	40.67±1.15 b	15.33±2.31c	10.67±1.15 de	7.33±1.15 c	33.33±2.31 d	
1/6 th of LC ₅₀	19.33±1.15 d	53.33±1.15 a	10.67±1.15 d	9.33±1.15 e	7.33±1.15 c	27.33±1.15 e	
Negative Control	98.00±0.00 a	2.00±0.00 f	0.00±0.00 e	0.00±0.00 f	0.00±0.00 d	0.00±0.00 f	
Positive Control	32.00±2.00 b	14.00±2.00 e	19.33±1.15 ab	14.67±1.15 bc	20.00±2.00 a	54.00±0.00 b	
Day 60 th of exposure							
1/3 rd of LC ₅₀	9.33±2.31 e	11.33±1.15 e	34.00±3.46 a	27.33±1.15 a	18.00±2.00 bc	79.33±1.15 a	
1/4 th of LC ₅₀	10.67±1.15 e	26.67±2.31 b	24.67±1.15 b	21.33±1.15 b	16.67±1.15 cd	62.67±1.15 b	
1/5 th of LC ₅₀	26.67±1.15 c	22.67±3.06 c	20.67±1.15 cd	15.33±1.15 de	14.67±1.15 d	50.67±3.06 d	
1/6 th of LC ₅₀	18.67±1.15 d	36.67±3.06 a	19.33±1.15 d	14.67±1.15 e	10.67±3.06 e	44.67±3.06 e	
Negative Control	98.00±0.00 a	2.00±0.00 f	0.00±0.00 e	0.00±0.00 f	0.00±0.00 f	0.00±0.00 f	
Positive Control	30.67±1.15 b	12.67±2.31 de	20.00±2.00 d	16.67±1.15 cde	20.00±2.00 ab	56.67±1.15 c	

The means with similar letters in a single column for each variable are statistically non-significant at $p < 0.05$.

Statistically significant ($p < 0.05$) increase in DNA damage was observed in the blood erythrocytes of common carp, *Cyprinus carpio* due to exposure of polluted water (Klobucar *et al.*, 2010). Mixture of pesticides (chlorpyrifos+endosulfan+thiram) has been reported to induce significantly higher DNA damage (Tope and Rogers, 2009). Highest DNA damage due to exposure of pesticides has been observed by Jors *et al.* (2007). Exposure of fish to sub-lethal concentrations of 1/4th LC₅₀, 1/2nd LC₅₀ and 3/4th of LC₅₀ of carbosulfan gave significantly ($p < 0.01$) higher DNA damage in erythrocytes and gill cells as compared to the control group. DNA damage in both the tissues was found to be dose and time dependent (Nwani *et al.*, 2010). Similarly, exposure of chlorpyrifos to the fish caused significantly higher DNA damage to the fish erythrocytes as compared to control groups, indicating genotoxic potential of this pesticide (Ali *et al.*, 2008). Sharma *et al.* (2007) also reported significantly higher DNA damage in *Mytus vittatus* exposed to different concentrations of endosulfan for 43 days as compared to positive control. Cavalcante *et al.* (2008) also reported significantly higher DNA damage in fish, *Prochilodus lineatus* exposed to 10mgL⁻¹ of roundup as compared to negative control. Dose and time dependent damage in DNA in the tissue of *Labeo rohita* exposed to organophosphate pesticide were also observed by Mohanty *et al.* (2011). Similarly, Kumar

et al. (2010) reported significantly higher DNA damage in fish (*Channa punctatus*) exposed to malathion than that of control groups. Dose dependent breakage of DNA caused by furadon, a carbamate pesticide, in the cells of *Labeo rohita* were also observed by Mohanty *et al.* (2013). The DNA damage increased significantly in erythrocytes of *Oreochromis niloticus* exposed to different concentrations of atrazine (Ventura *et al.*, 2008).

The DNA damage detected in the present study could have originated from DNA single strand breaks, double strand breaks, DNA-DNA/DNA-protein cross linking or inhibition of the enzymes involved in DNA repair resulting from the interaction of pesticides or their metabolites with DNA (Guilherme *et al.*, 2012). Chronic exposure of mutagenic pollutants leads to accelerated DNA strand breaks (Ternjej *et al.*, 2010) because DNA repair capacity of fish cells is low as compared to other animals (Kienzler *et al.*, 2013).

Marinowic *et al.* (2012) also reported significantly higher DNA damage index than that of control group of fish (*Bryconamericus iheringii*) exposed to, beta-cyfluthrin insecticide. The increase in DNA damage due to glyphosate has been reported in *Carassius auratus* and *Oreochromis niloticus* by Cavas and Konen (2007 and 2008) also. Species specific variations in DNA damage in peripheral erythrocytes of *Labeo rohita* were observed.

Fish exhibited significantly ($p < 0.01$) higher DNA damage due to endosulfan exposure, which induce larger comet tail lengths (Nagpur *et al.*, 2008).

Genotoxicity not only reduces the fitness of fish populations but also causes risk to the humans through the food web (Kapour and Nagpure, 2005). The present study reveals that $1/3^{\text{rd}}$ LC_{50} exposure of pesticides mixture to fish caused significantly higher DNA damage while negative control exerted significantly least damage to the nuclei. In conclusion, the exposure of aquatic life to pesticides means a constant health hazard for the population. So, human population is at massive risk by consuming these toxicated fishes. This implies that we should be careful in the application of pesticides to protect the life of fish.

ACKNOWLEDGEMENT

The author is grateful to the Higher Education Commission, Pakistan for providing funds under the Indigenous Ph.D. Fellowship Program (Pin# 117-4654-BM7-094) to complete this work as a part of Ph.D.

Statement of conflict of interest

There is no conflict of interest

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Nuclear Abnormalities in Erythrocytes of *Cyprinus carpio* under the Exposure of Metals Mixture

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ABSTRACT

Heavy metals present on earth are known due to their detrimental and noxious effects on the life of human beings as well as on aquatic organisms. Limited efforts have been done in the past to evaluate the genotoxicity of metals mixture in commercially important fish, *Cyprinus carpio*. Therefore, the main objective of this work was to assess the dose dependent genotoxicity of three metals in the form of mixture (Pb-Cd-Co) to *Cyprinus carpio* by using micronucleus (MN) assay at constant laboratory conditions. Genotoxic potential of metals mixture was determined in terms of nuclear abnormalities under sublethal concentrations exposure of Pb-Cd-Co for the period of one month. *Cyprinus carpio* were exposed to treatments (sublethal concentrations of metals mixture) viz. 1/3rd, 1/4th, 1/5th, 1/6th and 1/7th of 96-hr LC₅₀, separately and compared with control. During one month of exposure period, fish blood were sampled on weekly basis and directly smeared on slides for micronucleus assay. Nuclear abnormalities (%) were scored in terms of bi-nucleated, dumbbell, blebbed, notched and deformed nuclei. Data were statistically analyzed and results articulated in the form of Means±SD. Means of data were compared to find the statistical difference via Duncan Multiple Range test. Ability of *Cyprinus carpio* to induce micronuclear abnormalities in blood erythrocytes varied significantly ($p < 0.05$). Dose based increase in micronuclei frequency was observed in peripheral blood cells of fish. Results showed that fish exhibited significantly variable response against different sublethal treatments of Pb-Cd-Co mixture.

Article Information

The article was presented in 39th Pakistan Congress of Zoology (International) held on 4-6th March 2019, organized by Islamia College University, Peshawar, Pakistan.

Authors' Contribution

BB performed experiment work. AF statistically Analyzed the data. RI wrote the article. SK helped in the write-up and proof reading of this manuscript.

Key words

Metals mixture, Carp, Micronuclei, Lead, Blood cells

INTRODUCTION

Contamination of aquatic environment due to heavy metals, disturb the ecological balance and population of aquatic species due to their non-biodegradable and accumulative nature (Farombi *et al.*, 2007; Fatima *et al.*, 2015). Unfortunately, metals in the form of mixture are present in water and there are increased chances of their interaction. This contamination of water cause health hazards and severely damages the life of aquatic organisms (Zhu *et al.*, 2011). Oxidative stress and carcinogenesis are those properties of metals which may cause the formation of free radicals of oxygen and proved to be injurious to fish health (Rashed, 2001).

Fish is among those vertebrates, that are widely consumed by humans because of its high protein content and omega-3 polyunsaturated fatty acids which stabilizes cholesterol levels and consequently prevent cardiovascular diseases (Vilizzi and Tarkan, 2016). Fish in comparison with other animals is highly sensitive to many toxicants (Ambreen and Javed, 2016). Studies on various fish species

showed that changes in the biochemical and functional properties of blood and tissues are also the results of heavy metals exposure (Basa and Rani, 2003). Therefore, fish widely used as bio-indicator of aquatic pollution.

Toxicity due to heavy metals can be estimated by performing toxicity trials by using concentration dependent association (Akhter *et al.*, 2008). Toxicity of metals also varies according to their nature i.e. essential and non-essential as well as structure of metals. Among heavy metals lead is the most persistent and highly hazardous metal. (Godwin, 2001). Blood erythrocytes of fish when subjected to even minor doses of Pb may results in various chromosomal abnormalities (Cestari *et al.*, 2004). Several blood parameters viz. nervous system damage, damage to erythrocytes and leucocytes are also because of chronic Pb exposure (El-Badawi, 2005). Cadmium being non-essential metal cause mutagenic, teratogenic and carcinogenic effects on aquatic organisms, especially on fish so aquatic organisms are more sensitive to cadmium (Burger, 2008). Exposure of cadmium also causes micronuclei induction in polychromatic erythrocytes of *Cyprinus carpio* (Zhu *et al.*, 2004). Cadmium also interferes with the enzymes and DNA repair systems (Jia *et al.*, 2011).

Cobalt is also persistent heavy metal which is beneficial for fish because it contains vitamin B12 (Ahilan

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0030-9923/2019/0039-0013 \$ 9.00/0
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and Jeyaseelan, 2001). However, exposure of highest concentrations of Co to fish, results in the loss of appetite and consequently less or no growth (Javed, 2013). Due to its ability to bio-magnify in the aquatic food chain and highly persistent nature cobalt toxicity is a serious threat to fish and other aquatic organisms (Ubaid-ullah *et al.*, 2004).

Micronuclei assay is considered as an excellent and quickest tool for assessing the genotoxicity of waterborne toxicants. Little fragments of chromatin portion that are formed from wrecked parts of chromosomes are known as micronuclei (Kousar and Javed, 2015). Therefore, present experiment was conducted to estimate the micronuclear abnormalities in erythrocytes of *Cyprinus carpio* under metals mixture exposure.

MATERIALS AND METHODS

Experimental conditions

The experiment on nuclear abnormalities in erythrocytes of *Cyprinus carpio* under the exposure of metals mixture was conducted in the laboratory of Department of Zoology, Govt. College Women University Faisalabad. The test organism was *Cyprinus carpio* that was tested against tertiary mixture of metals i.e. Pb-Cd-Co. The fish fingerlings (180-days old) were obtained from the Government Fish Seed Hatchery, Faisalabad and brought to the laboratory. Fish were acclimated to laboratory conditions for two weeks and fed with crumbled feed (30% digestible protein and 2.70 K cal g⁻¹ digestible energy). After acclimatization, healthy fish of similar weight were selected for the experiment. The glass aquaria were washed thoroughly and filled with 50 liter dechlorinated tap water.

Stock solutions

Desired weight of chemically pure chloride compounds (MERCK) of lead, cadmium and cobalt were dissolved, separately, in 1000ml deionized water and stock solutions prepared for each metal. However, the desired metals mixture concentrations were prepared on ion equivalence basis by mixing the individual stock solutions.

Treatments

Cyprinus carpio was exposed to sub-lethal concentrations (1/3rd, 1/4th, 1/5th, 1/6th and 1/7th) of 96-h LC₅₀ of tertiary metals mixture determined by Ambreen (2016). *Cyprinus carpio* (n=10) were kept in aquaria for each test concentration of metals mixture, separately along with control group. Each concentration was tested with three replications. Aquarium water were renewed and maintained with desired concentration after every 24-hours to prevent any lowering of exposed concentration. To avoid stress on

the fish, the half sub-lethal concentration of mixture was added within 3 h and full toxicant concentration in 6 h. Test media were supplied with constant air flow with an air pump. The pH (7.75), total hardness (225 mg L⁻¹) and temperature (30° C) of water were maintained throughout the experiment of 30 days. Fish were fed with pelleted diet twice a day throughout the experimental duration. Physico-chemical variables of water viz. dissolved oxygen, carbon dioxide, total hardness, total ammonia, calcium and magnesium in the test mediums were determined by following the methods of A.P.H.A. (1998).

Micronucleus assay

A drop of blood from the fish caudal vein was directly smeared on slide and air-dried. Smears were subsequently fixed in methanol for 10 min and stained with Wright-Giemsa stain for 8 min (Barsiene *et al.*, 2004). The composition of stain was as: Wright's stain powder (300mg), Giemsa stain powder (30mg) and absolute methyl alcohol (100ml). The frequency of micronuclei, nuclear buds and bi-nucleated erythrocytes were evaluated (per 1,000 cells) by scoring at a 1,000 X magnification by using a trinocular microscope (EUROMEX Bioblue Holland) under oil emersion (100 X) lens. A total of 1,000 erythrocytes with intact cellular and nuclear membranes were examined for each fish species both for control and sublethal treatments. Blind scoring of micronuclei and other nuclear abnormalities of cells (binucleated, dumbbell, notched, blebbed and deshape of cells nuclei) were performed on coded slides. Round or ovoid-shaped non-refractory particles with the colour and structure similar to chromatin, with a diameter equaling 1/3rd or less of the main nucleus and clearly detached from it were interpreted as micronuclei. In general, the color intensity of MN was the same or lower than that of the main nuclei using criteria described by Fenech *et al.* (2003). Micronuclei frequency was calculated by using the following formula:

$$\text{Micronucleus frequency (\%)} = \frac{\text{Number of cells with micronuclei}}{\text{Total number of cells counted}} \times 100$$

Statistical analyses

Factorial experiment with the three replications for each test concentration was performed to find-out statistical differences among various parameters. The treatment means were compared by using Tukey's/Student Newman-Keul test while the relationships among different parameters were determined by using regression and correlation methods (Steel *et al.*, 1996).

RESULTS

Table I shows the frequency of various nuclear abnormalities viz. micronuclei, binucleated, dumbled, blebbed, notched and deshaped nuclei in peripheral blood cells of *Cyprinus carpio* exposed to sublethal treatments of metals mixture (Pb-Cd-Co) along with control during 1st week of experiment. Among various sub lethal treatments ($1/3^{\text{rd}}$, $1/4^{\text{th}}$, $1/5^{\text{th}}$, $1/6^{\text{th}}$, $1/7^{\text{th}}$ of LC_{50}), the $1/3^{\text{rd}}$ level has induced significantly higher number of micronuclei with the mean frequency of $3.70 \pm 0.60\%$, followed by that of $1/4^{\text{th}}$, $1/5^{\text{th}}$, $1/6^{\text{th}}$, and $1/7^{\text{th}}$ of LC_{50} and control. Frequencies of all other nuclear abnormalities were also higher at $1/3^{\text{rd}}$ of LC_{50} exposure of mixture. Frequency of other nuclear abnormalities in terms of dumbled, blebbed, notched and deshaped also increased in dose dependent manner as compared to control group. Overall means exhibited that concentration dependent increase in the frequency of nuclear abnormalities with highest damage occurred due to $1/3^{\text{rd}}$ of LC_{50} followed by that of $1/4^{\text{th}}$, $1/5^{\text{th}}$, $1/6^{\text{th}}$, $1/7^{\text{th}}$ of LC_{50} of metals mixture (Table I).

Frequency of micronuclei and other nuclear abnormalities in blood cells of tested fish under sublethal treatments of metals mixture during 2nd week of exposure period varied significantly. Under different sublethal treatments of mixture, the frequency of micronuclei, dumbled, blebbed, notched and deshaped nuclei were significantly ($p < 0.05$) higher at $1/3^{\text{rd}}$ of LC_{50} as compared to control. However, frequency of binucleated nuclei ($6.50 \pm 1.00\%$) were observed higher due to $1/4^{\text{th}}$ of LC_{50} exposure, followed by $1/3^{\text{rd}}$, $1/6^{\text{th}}$, $1/5^{\text{th}}$ and $1/7^{\text{th}}$ of LC_{50} . Overall means exhibited concentration dependent increase in the frequency of nuclear abnormalities during 2nd week of experiment.

During 3rd week of experiment, variable frequencies of nuclear abnormalities were observed in erythrocytes of *Cyprinus carpio*. Exposure of $1/3^{\text{rd}}$ of LC_{50} of Pb-Cd-Co mixture caused significantly ($p < 0.05$) highest frequency of micronuclei ($4.40 \pm 0.10\%$) while it was significantly lowest ($0.30 \pm 0.06\%$) in control group fish. Nuclear abnormalities in terms of binucleated, dumbled and blebbed nuclei also showed concomitant increase in their frequency with increasing sublethal dose of metals mixture (from $1/7^{\text{th}}$ to $1/3^{\text{rd}}$). However, frequency of notched nuclei ($5.80 \pm 0.20\%$) were higher at $1/6^{\text{th}}$ of LC_{50} while $1/5^{\text{th}}$ of LC_{50} induce higher frequency of deshaped nuclei, followed by $1/3^{\text{rd}}$, $1/6^{\text{th}}$ and $1/4^{\text{th}}$ of LC_{50} .

During 4th week of exposure, various concentrations of metals mixture caused significantly variable induction of micronuclei and binuclei in blood cell that followed the order: $1/3^{\text{rd}} > 1/4^{\text{th}} > 1/5^{\text{th}} > 1/6^{\text{th}} > 1/7^{\text{th}} > \text{control}$ and; $1/4^{\text{th}} > 1/6^{\text{th}} > 1/3^{\text{rd}} > 1/7^{\text{th}} > 1/5^{\text{th}} > \text{control}$, respectively. Frequency

of blebbed nuclei was significantly higher in erythrocytes of fish due to $1/4^{\text{th}}$ of LC_{50} exposure, while frequency of the same was significantly lower in control fish. Frequency of dumbled ($11.40 \pm 0.20\%$) and nuclei were observed significantly higher at $1/3^{\text{rd}}$ of LC_{50} . It is evident from the Table I that significantly lower frequencies of all types of nuclear abnormalities were observed in erythrocytes of control fish.

Mixture exposure to fish caused significantly variable induction of nuclear abnormalities that followed the order: blebbed > deshaped > notched \geq micronuclei > binucleated > dumbled during 3rd week of exposure period (Fig. 1).

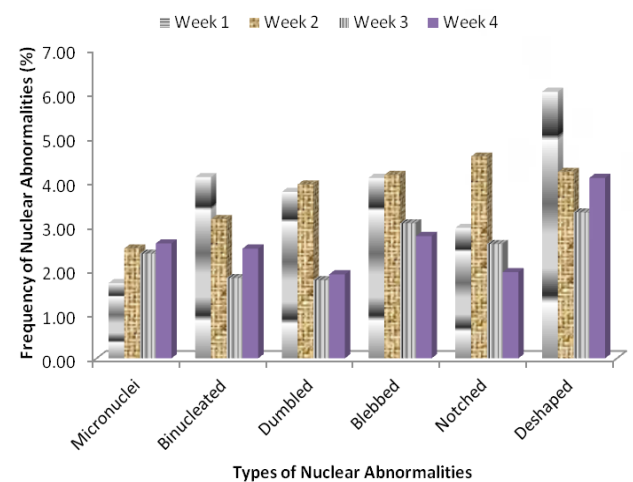


Fig. 1. Comparison of nuclear abnormalities in erythrocytes of *Cyprinus carpio*.

DISCUSSION

Due to industrial expansion, variety of chemicals, including metals, pesticides, hydrocarbons, organic and inorganic pollutants are released into the aquatic environments of Pakistan (Jabeen *et al.*, 2012) which not only disturb the physico-chemical properties of the water but also influence the aquatic food web to cause physiological and cytogenetic alterations in the aquatic animals, especially on fish (Barbosa *et al.*, 2009). Present results exhibited that among various sublethal treatments ($1/3^{\text{rd}}$, $1/4^{\text{th}}$, $1/5^{\text{th}}$, $1/6^{\text{th}}$, $1/7^{\text{th}}$ of LC_{50}), the $1/3^{\text{rd}}$ level induce significantly higher frequency of micronuclei, followed by that of $1/4^{\text{th}}$, $1/5^{\text{th}}$, $1/6^{\text{th}}$, $1/7^{\text{th}}$ of LC_{50} as compared to control. Frequency of other nuclear abnormalities in terms of dumbled, blebbed, notched and deshaped also exhibited dose dependent increase in their frequency as compared to control group. Metals in the form of mixture not only impair or harm the DNA of organism but they also adversely affect the shape and structure of blood erythrocytes

Table I. Frequency of micronuclei and other nuclear abnormalities in blood cells of *Cyprinus carpio* observed during 1st week of metals mixture exposure.

Duration	Sublethal Treatments	Frequency (%) of nuclear abnormalities						Overall means± SD
		Micronuclei	Binucleated	Dumbled	Blebbled	Notched	Deshaped	
1 st Week	Control	0.30±0.12f	0.10±0.06f	0.10±0.06f	0.20±0.06f	0.00±0.00e	0.20±0.12f	0.15±0.10f
	1/3 rd LC ₅₀	3.70±0.60a	10.30±0.10a	10.30±0.30a	6.80±0.10a	7.40±0.20a	10.60±0.60a	8.18±2.74a
	1/4 th LC ₅₀	2.20±0.60b	6.70±0.70b	4.60±0.30b	4.60±1.00c	3.30±0.30b	9.80±0.10b	5.20±2.71b
	1/5 th LC ₅₀	1.90±0.30c	2.70±0.10d	3.40±0.20c	6.20±0.20b	2.90±0.10c	7.80±0.20c	4.15±2.32 c
	1/6 th LC ₅₀	1.20±0.20d	3.30±0.30c	2.60±1.00d	4.10±0.10cd	2.80±0.20c	4.30±0.20d	3.05±1.13 d
	1/7 th LC ₅₀	0.90±0.30e	1.50±0.50e	1.60±0.20e	2.60±0.30e	1.30±0.30d	3.50±0.20e	1.90±0.97e
	Control	0.20±0.11f	0.00±0.00f	0.00±0.00f	0.10±0.50f	0.10±0.50e	0.00±0.00f	0.07±0.08 e
2 nd Week	1/3 rd LC ₅₀	4.30±0.20a	4.60±3.00b	8.60±1.00a	10.20±1.00a	8.20±10.00a	9.80±1.00a	7.62±2.56a
	1/4 th LC ₅₀	3.80±0.10b	6.50±1.00a	5.80±1.00b	5.40±2.00b	8.10±1.00a	5.90±1.00b	5.92±1.40b
	1/5 th LC ₅₀	3.10±0.20c	2.20±1.00d	4.20±0.60c	3.20±1.00d	4.20±0.20c	4.10±0.10c	3.50±0.81c
	1/6 th LC ₅₀	2.30±0.30d	4.40±0.30bc	2.80±0.20d	4.10±0.20c	5.30±0.30b	2.30±1.00e	3.53±1.25c
	1/7 th LC ₅₀	1.20±0.20e	1.20±0.20e	2.20±0.20e	1.90±0.10e	1.50±0.50d	3.20±0.20d	1.87±0.76d
3 rd Week	Control	0.30±0.06f	0.30±0.06f	0.00±0.00f	0.00±0.00f	0.20±0.06f	0.10±0.06f	0.15±0.14e
	1/3 rd LC ₅₀	4.40±0.10a	3.20±0.10a	3.10±0.10a	4.00±0.10a	2.90±0.30c	6.40±0.20a	4.00±1.31a
	1/4 th LC ₅₀	3.90±0.90b	2.60±0.20b	2.50±0.20b	3.90±0.20ab	1.40±0.20de	3.00±0.20d	2.88±0.95 c
	1/5 th LC ₅₀	2.50±0.50c	2.40±0.20bc	1.90±0.10c	3.83±0.25bc	3.60±0.10b	3.80±0.20b	3.01±0.84b
	1/6 th LC ₅₀	1.90±0.30d	1.40±0.20d	1.80±0.20cd	3.20±0.10e	5.80±0.20a	4.30±0.20c	3.07±1.72 b
	1/7 th LC ₅₀	1.20±0.20e	1.00±1.00e	1.30±0.30e	3.40±2.00d	1.60±0.20d	2.20±0.10e	1.78±0.90 d
4 th Week	Control	0.30±0.12f	0.10±0.06f	0.00±0.00e	0.20±0.06f	0.00±0.00d	0.10±0.06d	0.12±1.12d
	1/3 rd LC ₅₀	5.50±1.00a	5.00±1.00a	4.60±0.20a	0.60±0.20e	3.30±0.10a	11.60±0.20a	4.60±3.64 b
	1/4 th LC ₅₀	4.30±0.20b	4.70±0.70b	1.40±0.20c	6.80±0.10a	2.90±0.10 b	3.90±0.10 b	5.67±1.81a
	1/5 th LC ₅₀	3.00±0.30c	2.70±1.00c	3.20±0.20b	3.60±0.20 c	2.80±0.10b	2.10±0.10c	4.75±0.51 b
	1/6 th LC ₅₀	1.80±0.20d	1.30±1.00d	1.40±0.20c	4.20±0.10b	1.30±0.10 c	3.30±0.10b	4.55±1.24b
	1/7 th LC ₅₀	0.70±0.10e	1.10±0.30d	0.80±0.20d	1.20±0.20d	1.40±0.20c	3.50±0.10b	2.82±1.04 c

*Means with similar letters in a single column for each variable during each week are statistically non-significant at $p < 0.05$.

that are responsible for micronuclei formation as examined by [Rashed \(2001\)](#).

Our research findings suggest that for the assessment of genotoxicity, micronucleus assay in blood erythrocytes is the most authentic technique. Significantly higher nuclear abnormalities viz. notched, lobed and blebbed nuclei in *Cyprinus carpio* was examined by [Mitkovska et al. \(2017\)](#). Being a vertebrate, fish is a best choice to estimate potential risks because they are able to mobilize and amass pollutants in their bodies from water ([Kousar and Javed, 2015](#)). Fish blood erythrocytes are of great concern as they are nucleated and therefore can be used to detect DNA damage through single cell gel electrophoresis ([Costa et al., 2008](#)). Metals in the form of mixture showed more toxicity as compared to single or individual metals.

The results indicate that 1/3rd of LC₅₀ treatment of

metals mixture induce significantly substantial damage to nuclei in contrast to control group. Concomitant enhanced damage to DNA in outlying blood cells of *Cyprinus carpio* are the results of pronounced concentrations of metal ions. Significantly ($p < 0.05$) increased frequency of DNA distortion in peripheral erythrocytes of *Cyprinus carpio* was also observed after cessation of metals exposure ([Ahmed et al., 2011](#)) as compared to control. [Kousar and Javed \(2015\)](#) evaluated the genetic impairment in red blood cells of fresh water carps during 30 days of metals exposure. Significantly damaged nuclei of different types like notched, blebbed, dumbled, binucleated and deshaped cells under different of sublethal metals mixture concentrations were noticed during present experiment. In the same way, [Pereira et al. \(2013\)](#) observed genotoxicity in *Danio rerio* subjected to aluminum and cadmium

exposure. Their results showed remarkably highest DNA damage ($p < 0.005$) due to cadmium and aluminium toxicity. Ahmad *et al.* (2010) also observed dose dependent increase in nuclei damage after long term cadmium exposure to *Anabas testudines*. Toxicity due to arsenic exposure to the fish species viz. *Carassius auratus* and *Channa punctatus* was observed by Kumar *et al.* (2013). Significantly higher incidence of micronuclei along with occurrence of nuclear damage in blood cells was observed after metal exposure. During this experiment, statistically significant ($p < 0.05$) nuclear divergence was observed in the form of binuclei, blebbed, deshaped, notched and dumbled shaped nuclei under the exposure of Pb-Cd-Co mixture.

Present results of increased micronuclei frequency under $1/3^{\text{rd}}$ sublethal exposure of metal mixture can be compared with the findings of Kousar and Javed (2014) whose work on the metals and mixture showed high genetic instability in blood cells of major carps. Significantly ($p < 0.005$) highest frequency of micronuclei was also observed under different sublethal exposures of Zn+Cd mixture. Toxicity caused by tertiary mixture of metals viz. Pb+Cd+Zn to *Misgurnus anguillicaudatus* was determined by Zhang *et al.* (2008) and observed dose dependent rise in DNA damage after one month exposure of tertiary metals mixture. Likewise, a clear concentration dependent increase in genetic damage in erythrocytes of *Oreochromis mossambicus* was observed by Ambreen (2016). He found a dose dependent relation between fish and different concentrations of metal. Investigation on the toxic properties of mixtures of metals on fish are very rare, so there was a dire need to estimate the metal concentration present in the form of mixture in aquatic environment. Hence, it is required to relate the metals mixture effects though clarifying the relations among metals that may cause antagonistic, additive or synergistic effects.

In conclusion, Pb-Cd-Co mixture showed significant erythrocytic nuclear abnormalities in the peripheral blood erythrocytes of *Cyprinus carpio* under sublethal concentrations ($1/3^{\text{rd}}$, $1/4^{\text{th}}$, $1/5^{\text{th}}$, $1/6^{\text{th}}$ and $1/7^{\text{th}}$) of mixture as compared to control. Frequency of erythrocytic nuclear abnormalities was increased concomitantly with increased concentration of mixture exhibiting the dose dependent increase in damage.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the financial support provided by the HEC under SRGP Project #1361. Authors are also thankful to GC Women University for providing facilities for carrying-out this research work.

Statement of conflict of interest

There is no conflict of interest.

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Toxicity of Synthetic Insecticides and Neem Oil against Bio-control Agents of Cotton Mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) under Lab Conditions

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ABSTRACT

Studies were carried out to find out the toxicity of four chemical insecticides viz. commando (97% DF), confidor (20% SL), lannate (40% SP), actara (25 WG) and neem oil at various concentrations against the larvae of *C. carnea*, adults of *B. suturalis* and *A. bambawalei* through residual contact under laboratory conditions. Surface treated bioassay method was used for the evaluation of toxicity levels of four synthetic insecticides and neem oil. All the four tested insecticides caused significant mortality of the beneficial bio-control agents of *P. solenopsis*. According to the measured values commando was the highly toxic and actara was moderately toxic insecticide to tested bio-control agents of *P. solenopsis*. Neem oil was found to be harmless to larvae of *C. carnea* ($LC_{50} = 247.06 \text{ ml.L}^{-1}$) and adults of *B. suturalis* ($LC_{50} = 144.35 \text{ ml.L}^{-1}$) but slightly harmful to adults of *A. bambawalei* at higher doses. Interestingly, *A. bambawalei* males ($LC_{50} = 22.19 \text{ ml.L}^{-1}$) were more susceptible to neem oil than females ($LC_{50} = 33.82 \text{ ml.L}^{-1}$). Based on LC_{50} values it was concluded that neem oil is safe to the bio-control agents of *P. solenopsis* and can be used in compatible with integrated pest management programs.

Article Information

The article was presented in 39th Pakistan Congress of Zoology (International) held on 4-6th March 2019, organized by Islamia College University, Peshawar, Pakistan.

Authors' Contribution

MMR designed and performed the experiments and wrote the manuscript. AK analyzed the data and helped in culturing the mealybug and beneficial insects.

Key words

Synthetic insecticides, Neem oil, Bio-control agents, *P. solenopsis*

INTRODUCTION

Mealybugs are small, soft-bodied, plant sucking insects and their common name is due to the waxy material which covers the bodies of adult females (Downie and Gullan, 2004; McKenzie, 1967; Miller, 1991). A new menace to cotton in Pakistan since year 2005 was identified as *P. solenopsis* (Hodgson *et al.*, 2008). This pest spread rapidly to all cotton growing regions of the country and has become the most devastating pest of cotton and many other crops of economic importance (Arif *et al.*, 2009; Abbas *et al.*, 2010).

Several species of parasitoids and predators attack *P. solenopsis* and under pesticide free conditions can effectively control mealybugs (Tanwar *et al.*, 2007; Gautam *et al.*, 2010; Ram and Saini, 2010). *Brumus suturalis* and *C. carnea* are generalist predators of cotton insect pests. Both species are also important predators of cotton mealybug and have been found to consume all its nymphal instars (Sattar *et al.*, 2007; Khuhro *et al.*, 2002). *Aenasius bambawalei* Hayat is a solitary endo parasitoid of *P. solenopsis* and have potential for use in

augmentative release programs for its suppression (Hayat, 2009; Ram and Saini, 2010). These bio-control agents contribute significantly to the biological control of cotton mealybug. Despite their role in the biological control, their sensitivity to the agro-chemicals and bio-chemicals have never been tested which are extensively used for the control of cotton mealybug.

The joint use of biological and selected agro-chemicals for the control of insect pests is one of the strategies for the IPM of insect pests because the mode of attack of these pests on crop is so complex which cannot be controlled only with the biological means. Control of cotton mealybug is relied upon the excessive use of broad spectrum synthetic insecticides but very little is known about the direct effects of these toxic chemicals used in cotton field on bio-control agents of cotton mealybug.

An experiment was conducted to investigate the compatibility of these bio-control agents with the synthetic insecticides and neem oil and to check the resistance or tolerance if any, of these natural enemies and beneficial agents against pesticides and neem oil.

MATERIALS AND METHODS

Studies were carried out in the laboratory of Entomology section, Agricultural Research Institute, Dera

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0030-9923/2019/0039-0021 \$ 9.00/0
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Ismail Khan to evaluate the toxicity levels of various synthetic insecticides and neem oil against bio-control agents of *P. solenopsis*.

Culture of Aenasius bambawalei

A stock colony of parasitoid, *Aenasius bambawalei* Hayat was maintained in the bio-control laboratory of Entomology Section, Agricultural Research Institute, Dera Ismail Khan. Adults of *A. bambawalei* were kept in clear plastic cages with polyester mesh on one side for ventilation. Honey and sugar solution was provided on small cards as an artificial diet for adult *A. bambawalei*. The fresh leaves of cotton crop infested with the adults of cotton mealybug (*P. solenopsis*) were placed daily in rearing cages of *A. bambawalei* and offered for parasitization. After 24 h the parasitized mealybugs were removed. After 4-6 days the mealybug adults turned into dark brown barrel shaped mummies confirming the parasitization of adult female mealybugs. Adults emerged from the parasitized adult mealybugs 7-12 days after the parasitization. Newly emerged adults (Male and Female) were used for bioassay experiments.

Culture of Chrysoperla carnea

Adults of *C. carnea* were collected from cotton fields in Dera Ismail Khan, Pakistan in June 2009 and reared in clear plastic jars of 30 x 30 x 30 cm³ with mesh on one side and were reared on artificial diet consisting of Yeast, honey and distilled water, mixed to a paste. The collected adults were provided with artificial diet in the form of droplets placed on a plastic sheet having size of 5x5 cm². The back side of jar was sealed with net cloth for ventilation. A black card sheet was installed inside on top of the cage to facilitate adult females of *C. carnea* for egg laying. The fresh eggs laid by adult females were collected on daily basis by using a razor blade. Newly hatched larvae of *C. carnea* were reared on eggs of *Sitotroga cerealella*.

Culture of Brumus suturalis

The *Brumus suturalis* adults and their host *P. solenopsis* required for the study were collected from cotton fields and were cultured in plastic cages (13 x 13 x 13 inches) having mesh cloth on one side to facilitate ventilation. The newly hatched larvae of *B. suturalis* were cultured on nymphs of *P. solenopsis*. The newly hatched larvae of *B. suturalis* were cultured in a rearing tray having single cells until emergence of adult beetles. Larvae were reared individually to prevent competition and cannibalism. Newly emerged adults were used for bioassay experiments.

Bioassay treatment

Surface treated bioassay method was used for the evaluation of toxicity levels of four synthetic insecticides viz. actara, confidor, commando plus and lannate and neem oil against 3 day old larvae of *C. carnea* and newly emerged adults of *B. suturalis* and *A. bambawalei*. These pesticides were selected due to their frequent use for mealybug control in cotton growing regions. Six concentrations of each insecticide and neem oil were tested against the mentioned bio-control agents of *P. solenopsis*. Same concentrations of each insecticide and neem oil was tested at the start of trial against all bio-control agents of *P. solenopsis* but since mortality of *C. carnea* was negligible at the tested doses so another trial of higher doses for *C. carnea* was started. Glass Petri dishes (9 cm diameter) were used for *C. carnea* whereas; transparent plastic jars (5 x 3 cm²) were used for *B. suturalis* and *A. bambawalei*. The testing arenas were thoroughly treated with various concentrations of each insecticide and neem oil then allowed for complete air drying for 30 minutes. Each treatment was replicated 5 times and contained eight larvae of *C. carnea* or adults of *B. suturalis* or *A. bambawalei* in each replication. The Petri dishes sprayed with distilled water alone served as untreated control. Larvae of *C. carnea* were introduced individually in the Petri dishes to prevent cannibalism between the larvae. The numbers of dead larvae or adults in each treatment were recorded after 24 and 48 h of exposure periods. During the trial, the larvae of *C. carnea* were fed on *Sitotroga cerealella* eggs and *P. solenopsis* were provided as food to adults of *B. suturalis*. Honey and water solution was provided as food on a plastic sheet for the parasitoids during the 48 h exposure period. In this way a total of 2480 adults of *A. bambawalei* and 1240 of *B. suturalis* and 1240 larvae of *C. carnea* were tested for bioassay in five replications. The tested arenas were kept in an incubator maintained at 30 ± 2°C with 65% R.H. and a photoperiod of 12:12 h (L:D). Mortality of these bio-control agents was recorded 24 and 48 h after exposure.

Extraction and preparation of neem oil concentrations

Neem seeds were collected during 2009 from neem plantation of Agricultural Research Institute, Dera Ismail Khan. Seed kernels were removed from the seed, shade dried and crude neem oil was extracted by crushing seeds in a double-screw oil expeller and stored in laboratory until used. The required concentrations were prepared from the stock solution by mixing calculated amount of distilled water following standard method described by Musabyimana *et al.* (2001).

Table I. Details of conventional insecticides and neem oil evaluated for bioassays against bio-control agents of cotton mealybug exposed on sprayed arenas.

Treatments	Active Ingredient	Recommended Dose	Test concentration ($\mu\text{l a.i.L}^{-1}$)
Actara 25 WG	Thiomethoxam	24 g.acre ⁻¹	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Confidor 20% SL	Imidachloprid	250 ml.acre ⁻¹	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Commando plus 97% DF	Acephate	300 g.acre ⁻¹	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Lannate 40% SP	Methomyl + thioacetimidate	250 g.acre ⁻¹	0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]
Neem oil	Azadirachtin		0.001, 0.01, 0.1, 1, 10, 100 [1, 10, 100, 1000, 10000, 100000]

Values in [] represent concentrations evaluated for bioassays against *C. carnea*.

Table II. LC₅₀ values of different synthetic insecticides and neem oil against *C. carnea* ^a (n = 5).

Insecticides	Exposure time	Slope \pm SE [Log ₁₀ (dose)]	LC ₅₀ (mL.L ⁻¹) (95% FL)
Actara 25WG	24 h	0.730 \pm 0.093	0.507 (0.507 – 0.508)
	48 h	0.547 \pm 0.069	0.049 (0.025 – 0.099)
Confidor 20% SL	24 h	0.505 \pm 0.602	0.16 (0.082 – 0.0340)
	48 h	0.370 \pm 0.052	0.021 (0.008 – 0.056)
Commando plus 97% DF	24 h	0.455 \pm 0.057	0.064 (0.001 – 0.003)
	48 h	0.474 \pm 0.063	0.022 (0.010 – 0.048)
Lannate 40% SP	24 h	0.565 \pm 0.069	0.034 (0.178 – 0.676)
	48 h	0.468 \pm 0.065	0.010 (0.004 – 0.024)
Neem oil	24 h	0.627 \pm 0.276	247.06 (58.47 – 10438.5)
	48 h	0.409 \pm 0.090	231.93 (64.13 – 287.39)

^a The LC₅₀ values were calculated using generalized linear models with binomial errors.

Statistical analysis

Lethal concentration (LC) estimates were estimated from insect mortality data using standard procedures (Pickett, 2009). The LC50 bioassay data were analyzed by using a generalized linear model with binomial errors (or quasibinomial if data were over dispersed) to find slope and its standard error at 5% level of probability. The “dose.p” function from the MASS library was used to find logit regression analysis estimated LC50 values and their standard errors (se). Using values of standard error 95% Confidence Intervals [CI; (LC50 \pm (1.96 \times se))] were calculated. Pair wise comparisons of LC50 values were significant at 1% level of probability if their respective 95% CI's did not overlap (Crawley, 2007).

RESULTS

Toxicity of insecticides and neem oil to *C. carnea* larvae

The results of toxicity of four insecticides and neem oil to larvae of *C. carnea* are presented in Table

II. Increase in concentration of insecticides linearly increased the proportion of mortality of *C. carnea* larvae. Commando (97% DF) was the most toxic insecticide for *C. carnea* (LC₅₀ = 0.064). The least toxic was the neem oil with LC₅₀ = 247.06. Lannate (40% SP) was the most toxic after 48 h (LC₅₀ = 0.010), whereas, neem oil was the least toxicity having LC₅₀ = 231.93 after 48 h of exposure time.

Toxicity of insecticides and neem oil to adults of *B. suturalis*

Commando plus proved to be the most toxic insecticide after 24 and 48 h of exposure periods having least LC₅₀ values (1.44 and 0.16) as compared to other treatments (Table III). Among the synthetic insecticides actara was moderately harmful (LC₅₀ = 13.97 and 4.76) after 24 and 48 h of exposure periods. Neem oil was harmless to adults of *B. suturalis*. The LC₅₀ values of neem oil (144.35 and 133.65) differed significantly as compared to other treatments.

Table III. LC₅₀ values of different synthetic insecticides and neem oil against *B. suturalis* ^a (n = 5).

Insecticides	Exposure time	Slope ± SE [Log ₁₀ (dose)]	LC ₅₀ (mL.L ⁻¹) (95% FL)
Actara 25WG	24 h	0.1575 ± 0.043	13.97(17.31 – 112.71)
	48 h	0.042 ± 0.034	4.76 (0.002 – 101.98)
Confidor 20% SL	24 h	0.402 ± 0.063	2.91 (9.91 – 85.64)
	48 h	0.381 ± 0.050	1.11 (0.47 – 2.615)
Commando plus 97% DF	24 h	0.341 ± 0.052	1.44 (4.64 – 45.107)
	48 h	0.290 ± 0.043	0.16 (0.059 – 0.474)
Lannate 40% SP	24 h	0.654 ± 0.117	6.16 (27.62 – 137.80)
	48 h	0.481 ± 0.062	2.41 (1.14 – 5.08)
Neem oil	24 h	9.7 ± 18390.0	144.35 (Inf-0)
	48 h	9.97 ± 17508.98	133.65 (Inf-0)

^a The LC₅₀ values were calculated using generalized linear models with binomial errors.

Table IV. LC₅₀ values of different synthetic insecticides and neem oil against *A. bambawalei* (Male) ^a (n = 5).

Insecticides	Exposure time	Slope ± SE [Log ₁₀ (dose)]	LC ₅₀ (mL.L ⁻¹) (95% FL)
Actara 25WG	24 h	0.293 ± 0.043	0.23 (0.083 – 0.06)
	48 h	0.277 ± 0.048	0.002 (0.006 – 0.013)
Confidor 20% SL	24 h	0.139 ± 0.036	0.027 (0.002 – 0.250)
	48 h	0.258 ± 0.048	0.001 (0.003 – 0.011)
Commando plus 97% DF	24 h	0.198 ± 0.038	0.258 (0.063 – 1.050)
	48 h	0.303 ± 0.047	0.015 (0.004 – 0.048)
Lannate 40% SP	24 h	0.355 ± 0.050	0.026 (0.010 – 0.068)
	48 h	0.274 ± 0.048	0.003 (0.0007 – 0.015)
Neem oil	24 h	0.214 ± 0.047	22.19 (16.53 - 201.70)
	48 h	0.151 ± 0.039	14.39 (5.22 - 196.79)

^a The LC₅₀ values were calculated using generalized linear models with binomial errors.

Toxicity of insecticides and neem oil to A. bambawalei (male)

The most toxic insecticides tested against adult *A. bambawalei* (male) were lannate and confidor (LC₅₀ = 0.026 and LC₅₀ = 0.027) compared to other insecticides (Table IV). The toxicity (LC₅₀) of these compounds was almost 3.5 times higher than that of neem oil whereas, neem oil was the least toxic against the adults of *A. bambawalei* (LC₅₀ = 22.19). After 48 h confidor was the most toxic (LC₅₀ = 0.001) however, there was no significant difference in LC₅₀ against the *A. bambawalei* compared to other treatments. Neem oil was the least toxic (LC₅₀ = 14.39).

Toxicity of insecticides and neem oil to A. bambawalei (Female)

Table V shows the differences between the treatments

in terms of their toxicity to *A. bambawalei* females. Adults of *A. bambawalei* were most susceptible to actara (LC₅₀ = 0.006) which differed non-significantly from other treatments except neem oil (LC₅₀ = 33.82). Confidor, actara and commando were consistently the most toxic insecticides when *A. bambawalei* was exposed after 48 h by residual contact (Table V). Based on LC₅₀ values, neem oil was the least toxic (LC₅₀ = 256.32) compared to other treatments.

DISCUSSION

Synthetic insecticides are used commonly against insect pest without considering their effects on the environment and bio-control agents. Present experiments were conducted to investigate the effects of insecticides that were determined to be toxic to the pest are likely to be

Table V. LC₅₀ values of different synthetic insecticides and neem oil against *A. bambawalei* (Female) ^a (n = 5).

Insecticides	Exposure time	Slope \pm SE [Log ₁₀ (dose)]	LC ₅₀ (mL.L ⁻¹) (95% FL)
Actara 25WG	24 h	0.253 \pm 0.041	0.006 ()
	48 h	0.305 \pm 0.045	0.057 (0.02 – 0.160)
Confidor 20% SL	24 h	0.342 \pm 0.048	2.90 (1.10 – 7.63)
	48 h	0.264 \pm 0.044	0.012 (0.003 – 0.045)
Commando plus 97% DF	24 h	0.188 \pm 0.038	2.66 (0.52 – 13.42)
	48 h	0.230 \pm 0.039	0.219 (0.06 – 0.756)
Lannate 40% SP	24 h	0.348 \pm 0.047	0.316 (0.12 – 0.771)
	48 h	0.390 \pm 0.052	0.059 (0.02 – 0.139)
Neem oil	24 h	0.213 \pm 0.055	33.82 (0.056 – 204.14)
	48 h	0.223 \pm 0.053	25.63 (38.18 – 224.75)

^a The LC₅₀ values were calculated using generalized linear models with binomial errors.

harmless to the natural enemies of cotton mealybug. Botanical insecticides are safe to bio-control agents and can be used as a better and much safer options of IPM systems in comparison to chemical insecticides (Copping and Menn, 2000; Mamoon-ur-Rashid *et al.*, 2016). The products prepared from different parts of neem plants have been reported harmless to bio-control agents and can be used in compatible with integrated management programs (Lowery and Isman, 1995; Naumann and Isman, 1996).

In the present studies, neem oil proved safer to the larvae of *C. carnea* and adults of *B. suturalis*. Even at higher doses neem oil caused minimum mortality after residual contract as compared to synthetic insecticides. Aggarwal and Brar (2006) reported that the mortality of the first instar larvae of *C. carnea* was not affected by any of the azadirachtin enriched formulations; however, synthetic insecticide (triazophos) induced very high mortality rates of all the three larval instars. Schuster and Stansly (2000) found that bifenthrin was toxic to eggs, larvae and adults of *Chrysoperla rufilabris*. Neem oil was harmless to adult beetle *B. suturalis*. Adults of *A. bambawalei* were vulnerable to neem oil compared to *C. carnea* and *B. suturalis*. However, LC₅₀ values for *A. bambawalei* (22.19 and 33.82 mL L⁻¹ for males and females) were significantly higher in comparison to synthetic insecticides. The lower mass of males might be a possible reason for their greater susceptibility. The males were found more susceptible to botanical insecticides. The length of tibia in *Cotesia plutellae* males treated with extracts of *Melia azedarach* was responsible for this susceptibility (Charleston *et al.*, 2005). The present results also agree with those reported by Aggarwal and Brar (2006), they documented that Neemazal at lower dose (200 mg. L⁻¹) did not affect the emergence of *Encarsia sofia* adults, but at higher doses (800 mg. L⁻¹) there was a significant reduction in adult

emergence. Stansly and Liu (1997) found that neem extract had little or no effect on *Encarsia pergandiella* emergence from *Bemisia argentifolii*. Stark *et al.* (1992) found that low doses of neem seed extracts were safe to hymenopteran parasitoids.

All the tested insecticides were found toxic after 48 h exposure period except thiomethoxam which was moderately toxic insecticide to bio-control agents of *P. solenopsis*. Thiomethoxam has also been reported by Naveed *et al.* (2010) as slightly harmful to beneficial insects and harmless to predatory mites. Similar findings were also reported by Nasreen *et al.* (2005) who found thiamethoxam as moderately toxic to larvae of *C. carnea* at lower concentration. Among the tested bio-control agents, *C. carnea* was found most tolerant to synthetic insecticides and neem oil as compared to other beneficial agents. Ishaaya and Casida (1981) reported that larvae of *C. carnea* possess high hydrolyzing potential for synthetic insecticides due to the presence of esterases which is co-related with the tolerance of these larvae to synthetic insecticides. These results proved that the increase of mealybug populations to pest status in Pakistan is mainly due to the disruption of beneficial bio-control agents of mealybug by the wide scale use of synthetic insecticides.

Laboratory experiments showed that neem oil was non-toxic to the adults of *A. bambawalei*, *B. suturalis* and larvae of *C. carnea* and may be incorporated into IPM programs for the control of *P. solenopsis*. In contrast, thiamethoxam, imidacloprid, acephate and methomyl + thioacetimidate were highly toxic to bio-control agents under laboratory conditions, although, such high rates of mortality might not be projected under field conditions. Additional studies are needed to estimate the effects of insecticides and sub lethal effects of neem oil against selected bio-control agents under field conditions.

ACKNOWLEDGEMENTS

We thank Ministry of Food and Agriculture for their financial support for the project “Biological Control of Major insect pests of Cotton including Mealybug in Pakistan, D.I. Khan component” to undertake this study.

Statement of conflict of interest

The authors declare there is no conflict of interest.

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Some Abstracts

PLENARY LECTURE-1**ENDOGENOUS OPIOID PEPTIDES MODULATE PANIC-LIKE EMOTIONS:
INTERACTIONS BETWEEN OPIOID AND GABAERGIC SYSTEMS****Norberto Cysne Coimbra***Ophidiarium LNN-FMRP-USP/INeC**Ribeirão Preto School of Medicine of the University of São Paulo (FMRP-USP)**Av. Bandeirantes, 3900, Ribeirão Preto, 14049-900, São Paulo, Brazil*

The effects of endogenous opioid peptide antagonists on panic-related responses are controversial. Using elevated mazes and a prey-versus-predator paradigm, we investigated the involvement of the endogenous opioid peptide-mediated system in the modulation of anxiety- and panic attack-induced responses and innate fear-induced antinociception in the present work. Wistar rats were intraperitoneally or intracollicularly treated with or naloxone at different doses and were subjected to either the elevated plus- or T-maze test or confronted by *Crotalus durissus terrificus*. The defensive behaviours of the rats were recorded in the presence of the predator and at 24 h after the confrontation, when the animals were placed in the experimental enclosure without the rattlesnake. The peripheral and intramesencephalic non-specific blockade of opioid receptors had a clear anxiolytic-like effect on the rats subjected to the elevated plus-maze but not on those subjected to the elevated T-maze; however, a clear panicolytic-like effect was observed, i.e., the defensive behaviours decreased, and the prey-versus-predator interaction responses evoked by the presence of the rattlesnakes increased. A similar effect was noted when the rats were exposed to the experimental context in the absence of the venomous snake. After completing all tests, the naloxone-treated groups exhibited less anxiety/fear-induced antinociception than the control group, as measured by the tail-flick test. These findings demonstrate the anxiolytic and panicolytic-like effects of opioid receptor blockade. Fearlessness was also recorded after microinjections of opioid receptor antagonists in ventral midbrain. The neural bases of opioid antagonists effect were also addressed by morphological approaches in independent groups of rats. Interactions between opioid links and neostriado-nigro-collicular GABAergic projections were demonstrated.

PLENARY LECTURE-2**APPLICATIONS OF CITIZEN SCIENCE, DATA MINING, AND SPATIAL CONCEPTS
IN ZOOLOGY AND AGRICULTURE****Awad Mohamed Awad Hassan****Department of Ecological Agriculture & Agri-Envrionmental Design, Szent Istvan University,
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Citizen science is the involvement of the public in scientific research – whether community-driven research or global investigations. The communication technology revolution, internet, and smartphone bandwidth have raised the suitability of citizen data to create science. From the other side, data mining is the practice of browsing and examining large pre-existing databases (mostly citizen data) in order to generate new information. Moreover, spatial concepts define the relationship between objects to each other. It depends on Geographic Information System (GIS), and mostly on Remotely Sensed data (RS), citizen data, and data mining methodologies. I have reviewed the available information about applications of citizen science, data mining, and spatial concepts in zoology and agriculture. The advantages, obstacles, and criticisms have been discussed. The current global situation, and the concurrent large projects and initiatives have been presented. Finally, some brain storming has been practiced to create expectations for these three methodologies in the future of zoology and agriculture science and applications.

PLENARY LECTURE-3**ZEBRAFISH: A VERSATILE MODEL FOR IN VIVO AND IN VITRO STUDIES****Muhammad Ibrahim**

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Use of animals in the lab is an important part of biological research. Animals can be used to test drugs, to understand gene function and to discover novel treatments etc. Zebrafish (a freshwater teleost fish) is emerging as a model species for various fields of biomedical research, for its ease of manipulation, availability of mutant lines and molecular similarities to humans. Zebrafish have been used to model various physiological and pathological conditions of humans. The embryos are often used in experiments. Zebrafish embryos are fertilized externally, optically transparent, and can be easily exposed to test substances. The fast development of zebrafish embryos allows data generation within five days after fertilization (until when the embryos are not considered as protected under animal welfare regulations). Several transgenic lines have been developed for zebrafish which express fluorescent proteins under cell specific promoters. These transgenic lines allow the tracking of the differentiation, proliferation and migration of individual cells during development in vivo and in vitro. Being originated from Pakistan, the fish has not given much attention as a laboratory animal in the country. We have developed kdrl zebrafish transgenic line as a model for studying vascular development in vivo and in vitro. Currently we are working on establishment of zebrafish facility at Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar under the HEC funded project.

PLENARY LECTURE-4**ZOOLOGICAL SOCIETY OF PAKISTAN — IN PURSUIT OF EXCELLENCE
(1968-2018)****Abdul Aziz Khan**

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The Zoological Society of Pakistan (ZSP) came into being on 3rd March 1968 for the promotion of science of Zoology and its allied branches, both pure and applied, through: (i) Holding of conferences, symposia and seminars (ii) Publication and distribution of a journal, monographs and books devoted to zoological research and education. In this study, 50 years' milestone activities and achievements of ZSP have been accessed and quantified. The first prime activity was to launch the publication of Pakistan Journal of Zoology (PJZ) which has been accomplished successfully with the completion of 50 volumes (1968-2018), containing 4,347 articles covering about 31,000 pages. Contribution of foreign authorship from China, Turkey and Saudi Arabia increased appreciably. Supplements of PJZ authored by senior Zoologists were encouraged and fully supported by ZSP. So far, 12 supplements to PJZ have been published covering 1,359 pages. PJZ is duly recognized, scientific journal by the Higher Education Commission (HEC), PCS&T, and an ISI impact factor publication. Also, the Society publishes the, "Proceedings of the Annual Pakistan Congress of Zoology". So far, 38 volumes have been published containing peer reviewed articles. The Second matchless activity of ZSP has been to organize and conduct "Pakistan Congress of Zoology" at different university campuses and research institutes. So far, 38 (1980-2018) such conferences, without any break, have been organized. The number of submitted and approved abstracts of presentations increased from 48 (1980) to 721 (2018) abstracts, showing tremendous participation of scientists and students. During these conferences 179 plenary lectures were presented by senior zoologists. Also, 10 symposia were organized on different topics of national importance. The most fascinating and

glorious part of the proceedings of a conference has been the presentation of award of Gold Medals, and appreciation certificates to senior zoologists, mid-career scientists and university position holder students. So far, 25 senior zoologists have been honored with Life-time Achievement Award. Similarly, 27 zoologists have been presented with Zoologist of the Year Award. Every year 5 Gold Medals are confirmed to mid-career scientists of zoology. So far, 42 Gold Medals (2001-2018) have been distributed. There are 12 Gold Medals instituted for university students. So far, 143 position holder students of MSc. and PhD. (Zoology) of different universities have received these medals. The society, with these salient achievements, has successfully accomplished the “Aims and objectives: as a mission through the commitment and enthusiasm of its fellows.

SECTION - I

CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY

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

1. HERBAL MEDICINE BIOCHEMISTRY, BIOTECHNOLOGY AND BIOINFORMATICS

EFFECT OF NATURAL AND COMMERCIAL FEED ON SERUM CHEMISTRY OF GREY PARTRIDGE (*PERDIX PERDIX*) IN CONFINEMENT REARING SYSTEMS

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Limited information exists about the effect of different diets on serum biochemistry of grey partridge (*Perdix perdix*) which reared in confinement rearing systems. In this study trial 40 research birds (grey partridge) chicks were grouped into 4 cages 10 chicks/cage. C₀ selected as control treatment and fed with diet containing millets other three groups were marked as C₁, C₂ and C₃ which were fed with different diets i.e. wheat/corn, herbs/insects and poultry feed respectively. Serum analyzed to check out the effect of different diets on cholesterol, total protein, Globulin, Creatinin, albumin, Uric acid, ALP, AST and ALT. Cholesterol, total protein, albumin, creatinin ALP values of birds in reared in C₂ and C₃ significantly (P<0.05) vary from C₀ birds serum while globulin, Uric acid, AST and ALT observed non-significant between C₁ and C₀ birds serum. The present shows that different diets affect the serum biochemical profile in grey partridge.

INVESTIGATION OF THE LIPID PROFILE IN TRANSFUSION DEPENDENT THALASSEMIC PATIENTS IN HYDERABAD, SINDH, PAKISTAN

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Thalassemia is chronic genetic disorder worldwide, which causes disturbance in blood hemolysis and anemia (destruction of red blood cells). For the management of hemoglobin level frequent blood transfusion affect the biochemical parameters in thalassemia patients. For assessment of the serum concentration of serum lipid profile blood was collected, total 174 including 84 thalassemia patients and 90 healthy persons (control). Serum lipid profile was measured by using international federation of clinical chemistry (IFCC) methods on Microlab 300. Data was computed by SPSS (version 22) and using Microsoft Excel 2010. We found lipid concentration significantly increase as compared to control, whereas total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C) were seen normal, triglyceride (TG) was also significantly increased, whereas in most of the patients TC:HDL level was significantly high, whereas HDL:LDL and TC: LDL were normal when compared with controls. (p <0.001). We found level concentration of triglycerides significantly high when compared with female TDT patient and female control, whereas HDL-C and LDL-C level was normal in both groups. Gender wise comparison of serum concentration of TC:HDL-C, HDL:LDL-C and TC:LDL-C in both group were normal when compared with controls. Age wise comparison with all age group TC:LDL-C were significantly high as compared to controls but we found no significant variation in HDL:LDL and TC: HDL among all age groups.

EVALUATION AND CORRELATION OF ANTI-OXIDATIVE AND ANTI-INFLAMMATORY RESPONSE OF CURCUMA LONGA (TURMERIC) IN ARTHRITIS PATIENTS RECEIVING CURCUMIN X4000

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Curcumin by integrity of its anti-inflammatory possessions assist with the soreness occurring in PMS and also stimulated brain chemicals and thus raised mood changes and depression. While there does not appear to be any harmful interactions after you take turmeric with any foods, varied herbs will increase your probability of excessive hurt and bruising. To estimate the anti-inflammatory and anti-oxidative response of Curcuma Longa in Arthritis Patients. Comparative Study. 5.0 ml Blood sample of 50 Arthritis Patients and 50 healthy/control persons was taken in gel clotted vial from orthopedic department of Mayo hospital and Jinnah Hospital Lahore. Estimation of MDA, GSH, CAT, SOD, Micronutrients (Vit.A, Vit.C, Vit.E) was done spectrophotometrically. MDA level in Arthritis patients was remarkably elevated (9.11 ± 0.32) than normal person (2.25 ± 0.21). The value of GSH in patients reduced (4.16 ± 0.45) as compared to normal individual (7.64 ± 0.88). The CAT level was decreased in patients (1.72 ± 0.53) while the value of healthy individual is high (3.19 ± 0.76). Results parade the amount of SOD in patients (2.31 ± 0.24) it was low in normal people (0.99 ± 0.24). Arthritis Patients has remarkably deficient vitamin A (73.99 ± 7.29) than normal person (98.25 ± 4.36). The outcome obtain revealed the worth of significant ($P=0.02 < 0.05$). Reduced level of Vitamin E in diseased person (1.15 ± 0.32) than normal (4.35 ± 0.79). So it depicts that arthritis patients shows the highly significant behavior ($P=0.00 < 0.05$). The present study expresses that Vitamins were also a factor as the arthritis is a bone disease so vitamin C and E also lacks in a patient's body. Turmeric as we know is an anti-inflammatory species so the intake of turmeric can reduce some of the amount of factors in the body causing inflammation.

THERAPEUTIC POTENTIAL OF CARDUS MARIANUM AGAINST HEPATIC CARCINOMA

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Cancer is a complex disease and the second major cause of death in human. Generally it is linked with accelerating effects both at the cellular and molecular levels. A different combination of surgery, radiation treatment, and chemotherapeutic means are involved for the management of cancer but still cancer remains associated with high mortality rate. Many medicinal plants have been reported for the management and treatment of numerous cancer and cancer-related disorders by the native herbalists. *C. marianum* is a medicinal plant which has been widely used in traditional European medicine. Now a day's silymarin, the purified extract of the fruits of *C. marianum*, are used in the treatment of diseases of the liver. N hexane, ethyl acetate and ethanol extracts of *C. marianum* were prepared by continuous hot extraction method using Soxhlet apparatus. All the extracts were assessed for their in-vitro cytotoxic effect on Human hepatocellular carcinoma cell lines (HepG-2 cell line) by MTT assay. Ethanolic extract exhibited the maximum cytotoxic effect (90.07%) at a concentration of 1000 µg/ml against the HepG-2 cell lines followed by ethyl acetate extract (82.22%) and n hexane extract (70.35%). The effective CTC50 value for ethanolic extract of *C. marianum* was found to be 141 µg/ml.

ANTICANCEROUS ACTIVITY ANALYSIS OF MARS DENIA CONDURANGO AGAINST HELA CELLS

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Condurango has been fruitfully used in various systems of complementary and alternative medicine (CAM) against esophageal and stomach ailments including certain types of cancer. However, until now a very less systematic

study has been conducted to verify its efficacy and dose with proper experimental support. Therefore, we evaluated its anticancer potential in-vitro in HeLa cells. Ethanolic extract of *Marsdenia Condurango* was prepared by soaking dried bark of condurango in ethanol for 14 days followed by filtration and evaporation using rotary evaporator. A (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay (MTT assay) was performed to check anticancerous potential of different doses of condurango against HeLa cells. The concentration inhibiting 50% of cell growth (IC₅₀) was calculated. *Marsdenia Condurango* exhibited pronounced effects on the HeLa cell line. Dose-dependent study revealed IC₅₀ of 0.27 mg/ml on the HeLa cells. The anticancer efficacy of an ethanolic extract of condurango against HeLa cells supports its use for treating cervical cancer.

SEROPREVALENCE OF TOXOPLASMOSIS AND ASSOCIATED RISK FACTORS IN FARMER'S FAMILY OF WAHGA BORDER, LAHORE, PAKISTAN

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Parasites are organisms that depend upon other organisms for food and shelter. Most of the humans carry several different kinds of parasites including both helminthes as well as protozoan. An important protozoan infection in tropical and subtropical regions is toxoplasmosis caused by *Toxoplasma gondii*, a worldwide infection and so as in Pakistan. It is expected to pass on a disease to 1/3rd of the people around the world. A study was designed to investigate the seroprevalence of toxoplasmosis in farmer's families of Wahga border, Lahore, Pakistan. Questioner survey was performed by interview while serological investigation was done by ELISA technique. About 90 samples were selected randomly for the detection of *Toxoplasma gondii* antibodies. Past infection of parasite was investigated by immunoglobulin IgG antibodies, detected by using a commercial enzyme immunoassay kit (ELISA). All these samples were categorized according to gender, age, socio- demographic factors and associated factors. For seroprevalence analysis, samples were randomly selected. In farmers family total seropositivity among selected samples was found to be 61.11%. Seropositive rate was highest in males as 75%, then in females as 54%, and in children as 40%. It has been concluded in this study that males were more exposed to toxoplasmosis than females and children. To prevent risk of toxoplasmosis from the environment, the contact with the soil should be avoided. The study recommends the need for further research in the whole country using different hematological and biochemical parameters.

STRUCTURAL INSIGHT INTO EGLB, AN ENDO-1,4-B-D-GLUCANASE OF *ASPERGILLUS FUMIGATUS* Z5 INVOLVED IN CELLULOSE BIODEGRADATION

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The most abundant biomass on Earth, cellulose is a polymer of glucose with β -1,4 linkages. Fungi of the genus *Aspergillus* are known to produce large amount of different cellulases to hydrolyze cellulose. Endoglucanase B (EC 3.2.1.4), which catalyzes the endohydrolysis of 1,4- β -D-glucosidic linkages in cellulose belongs to the large glycoside hydrolase family 5 (GH5). EglB of *Aspergillus fumigatus* z5 comprises of 329 amino acid residues (GenBank KMK63077) where residues 1–18 was signal sequence with cleavage site between Ala¹⁸ and Ala¹⁹. The sequence was 76.6 % identical with EglB of *A. niger* BRC31494 with fully resolved tertiary structure (PDB 5178) at 1.58 Å. The protein had molecular weight of 35.97 kDa with theoretical pI 4.70, aliphatic index (AI) 77.17, unfoldability 0.166 and molar attenuation coefficient (ϵ) 7995 M⁻¹ cm⁻¹ at 280 nm. EglB folds into two overall regions: one with 5 α -helices and a 3₁₀ helix and other with 3 α -helices and a 3₁₀ helix. Cys²³⁵ and Cys²⁷² form a disulfide bond. The results obtained using biological computation tools indicated that the protein was hydrophilic in nature, stable *in vitro* and had very low disorder probability. As shown by ϕ - ψ plot, about 91.2 % of the residues (300 of 329) lie in the most favored or core regions that confirmed good quality of the proposed tertiary structure. Based on the quality score, the proposed secondary and tertiary structures were correct and extremely good to represent EglB of *A. fumigatus* z5.

SPORE-SPECIFIC CATALASE, CATA, OF A PATHOGENIC DIMORPHIC FUNGI *HISTOPLASMA CAPSULATUM* NAM1 FOR H₂O₂-MEDIATED OXIDATIVE STRESS RESISTANCE

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Histoplasma capsulatum, the dimorphic pathogenic fungus of family *Ajellomycetaceae* is the most common cause of fungal respiratory infections (histoplasmosis) in healthy and immunocompromised individuals worldwide. *H. capsulatum* encounters oxidative stress during the earliest phase of infection as well as in the infection process due to the production of reactive oxygen species (ROS) like hydrogen peroxide (H₂O₂) upon the host inflammatory response. The enzyme catalase that converts H₂O₂ to water and molecular oxygen is the key component of oxidative stress tolerance in *H. capsulatum* like other dimorphic fungi. A catalase (GenBank EDN08610) comprised of 749 amino acid residues (84.69 kDa) was located in *H. capsulatum* Nam1, which has 70.9 % sequence identity with spore-specific monofunctional catalase CatA (XP747688) of *Aspergillus fumigatus* Af293. Majority of the identified accessory proteins of CatA of *H. capsulatum* were superoxide dismutases, which help to break down potentially harmful oxygen molecules in cells. Seven highly conserved residues in the protein that form the binding site were His⁹³, Ser¹³², Asn¹⁶⁶, Phe¹⁷¹, Phe¹⁷⁹, Arg³⁷⁶ and Trp³⁸⁰. Phylogenetic analysis revealed that catalase of *H. capsulatum* was evolutionary related to CatA of pathogens of lower animals of *Ajellomycetaceae* family *Emmonsia crescens* (PGH34163) and *Blastomyces parvus* (PGG96731) with 88.9 and 87.8 % sequence identity, respectively.

TRADITIONAL USES OF MEDICINAL PLANTS AGAINST ANIMAL DISEASE IN BAHAWALPUR REGION

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Present study was focused to record traditional uses of medicinal plants against animal disease in Bahawalpur. Survey was conducted through well-structured performa and a list of medicinal plants compiled. Total 61 species of medicinal plants belonging to 53 genera and 31 families were recorded and also identified the use of these plants against treatments of different diseases in livestock and human. Presently it was recorded that the use of leaves (42%) of the medicinal plants were most common and followed by fruits and seeds (12%) of each, root or rhizome (8%), whole plant (6%), bark (5%), flowers (3%) and other parts (12%) against different animal diseases. The traditional medicinal plant preparations were made by different methods including crushing, grinding, boiling, cooking, taken as raw, juice and some others. Most of the people used crushing and grinding methods for preparing medicine for treating different animal diseases. The percentage of crushing and grinding methods was 36% followed by taken as raw 24%, juice or liquid 16%, cooking or boiling 12% and some other methods make 12% of total preparations. These preparations were applied through different routes of administration like dermal, oral, nasal, ocular and otic routes. Oral route for the utilization of these plants was the most common (56%) and followed by dermal (37%), nasal (3%), ocular (3%) and otic routes (1%). The study revealed that the traditional healers and some livestock owners had knowledge of medicinal plants use to treat different animal diseases. Hence, further research is required to evaluate the efficacy of local medicinal plants and to determine the safe dosages for treatments.

EFFECT OF FISH MEAL REPLACEMENT WITH PLANT PROTEIN AND TAURINE SUPPLEMENTATION ON HEPATIC HISTOLOGY, INTERMEDIARY METABOLISM AND ENZYMATIC ACTIVITIES OF BLACK SEA BREAM JUVENILES

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Soybean meal is a reliable feed component being easily available worldwide, that is considered as a protein supplement in aqua nutrition. An 8-week experiment was conducted to study the effect the fish meal replacement with a soy protein in addition with taurine. Hepatic histology, intermediate metabolism and enzymatic activities were investigated. Four diets containing similar protein and energy level were prepared with 60% or 100% of fish meal replacement level added with or not with taurine. HE staining revealed in juveniles fed with control diet expressed normal structure possessing hepatocytes and sinusoidal spaces. Plant protein addition at 60% showed macrophages having a brown pigment while taurine addition at a similar SPC level showed enlarged hepatocytes having congested central venules swollen sinusoids and enlarged inter hepatic plate spaces and endothelial cells. Phosphofructokinase significantly increased in 100% treatment, having an effect between Phosphofructokinase and the taurine addition. Hexokinase (HK) activity showed no any significant difference among the dietary groups. Total protein in the liver was significantly affected in the complete replacement group when compared with the control diet. Taurine addition reduced the malondialdehyde activity in the SPC incorporated diets, however complete replacement group higher MDA activity when compared to the control diet. Total bile acid (TBA) showed no any significant difference among the dietary treatments. In conclusion, FM replacement by dietary Soy protein depressed the hepatic intermediate metabolism, while taurine supplementation restored the activity of the enzymes of *Acanthopagrus schlegelii* juveniles.

HONEY BEE WAX: A NATURAL HEALING APPROACH FOR CRACKED HEEL

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Heel cracks is considered one of the common problems in our environment. There are several scientific and homemade remedies are applied for the treatment of this problem. The cream was tested on the patients having the problem of heel crack of different age groups in various parts of Kohat city. To check the performance of the cream statistically, the proportion of cured patients were tested for significance through the proportion test of significance. The test result showed that a significant proportion of the sample is cured successfully. Out of 30 patients 16 (53%) were fully cured, 8(27%) were partially cured and 6(20%) were remained uncured. One of the reasons of uncured may be the severity of the problem because in those patients the problem was very severe. The prime objective of this study was to develop a new easily available treatment for this common problem. Therefore, we have developed a new cream from olive oil and beeswax for the treatment of heel cracks.

USE OF HONEY, BEE WAX AND OLIVE OIL AGAINST SKIN DISEASE MELASMA

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Melasma is common acquired pigmentary disease characterized by symmetric hyper pigmented patches, mostly commonly seen in females face. Multifactor are responsible for their Implicated in its etiopathogenetic, mainly

sunlight, iron and other micronutrient deficiency, pregnancy and thyroid disorder. In our study we prepare a mixture of bee wax, olive oil and honey in water bath with 1: 3: 1/2. A significant reduction in melasma among Honey, bee wax and olive oil using patients. Out of 25 patients 15% were fully cured, 7% were partially cured and 3% were not cured due to a lot of internal problems. Results show that application of natural products honey, bee wax and olive oil is effective in managing melasma. The aim of the study is to evaluate the effect of natural products Honey, bee wax and virgin olive oil and melasma. It was concluded from the current study that the natural honey and their product have significant importance toward the described disease.

PRODUCTION OF GLUCOAMYLASE BY FUNGAL CONSORTIUM USING SUBMERGED FERMENTATION

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The glucomylase has enormous number of applications in different industries including textile, food and detergents etc. It is also use in the production of fructose syrup, dextrose, and agrochemicals. The present study deals with isolation, screening and optimization of cultural conditions for the production of glucoamylase from fungal consortium. Different fungal strains having amylolytic potential were isolated from different sources like soil, bread, fruits and vegetables etc. Primary screening was carried on the basis of starch hydrolysis zone. All the isolated strains were subjected to compatibility testing. Compatible strains were screened for the production of glucoamylase through submerged fermentation. The consortium No.12 which was identified as *Alternaria* sp and *Aspergillus niger* gave highest glucoamylase production. Effect of media on the production of glucoamylase was also investigated. The M4 medium containing g/L Starch 20, KH₂PO₄ 14, NH₄NO₃ 10, KCl 0.5, MgSO₄·7H₂O 0.1, NaNO₃ 10, FeSO₄·7H₂O 0.01 gave maximum production. The physical and nutritional factors including temperature, pH, rate of fermentation, carbon and nitrogen sources, inoculum size, volume of media, metal ions, and surfactants were also optimized for maximum production of glucoamylase. The maximum production was obtained after 72 hours of incubation at 30°C, pH 5.5, inoculum size 4ml, and 25ml fermentation medium. 1% maltose and 2.5% sodium nitrate were optimized as carbon and nitrogen sources, respectively. Zinc sulphate (1%) and Tween 80 (1%) were optimized as suitable metal ion and surfactant for the maximum production of glucoamylase.

BACTERIAL SYNTHESIS OF RIBOFLAVIN: SCREENING AND OPTIMIZATION OF PHYSICOCHEMICAL CONDITIONS

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Vitamins are important factors to carry out essential functions of the body like formation of nucleic acids, antioxidant activities and cell metabolism. Mostly, vitamins are present in a variety of foods, but instead of this vitamin deficiencies in humans still prevail in many countries, just because of insufficient food intake and unbalanced diets. Microorganisms are used to produce valuable molecules including vitamins. Vitamins can be produced at large scale by using different microorganisms. The present work was carried out to isolate and screen new riboflavin producing bacteria. Bacterial isolates were isolated from soil by dilution spread method. Upon secondary screening by riboflavin assay method, out of 17 isolates one isolate Fs8 was selected that produced highest amount 0.872mg/ml of riboflavin. Gram staining revealed Gram positive cocci. Different optimization parameters were considered like temperature, pH, carbon sources, aeration and non aeration conditions, inoculum size and days for riboflavin production. Optimized temperature was 55°C because at this temperature Gram positive cocci bacteria produced maximum riboflavin 0.11mg/ml. Optimized pH was 6.5 because at this pH gram positive cocci bacteria

produced maximum 0.019mg/ml riboflavin. Optimized carbon source was glucose because it gave best riboflavin production which was 0.006 mg/ml. Aeration condition is optimized because it produced maximum riboflavin 0.013mg/ml. Different inoculum sizes were considered and out of this best quantity of riboflavin was observed at inoculum size 10^4 ml. Further analyzing the growth conditions the microbe has potential application in industry.

INVESTIGATION OF THE INTERPLAY BETWEEN O-PHOSPHORYLATION AND O-GLCNACYLATION IN REGULATION OF THE VITAMIN D RECEPTOR, IN SILICO

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The vitamin D receptor (VDR) binds to vitamin D and other ligands to control and maintain calcium and phosphate homeostasis, bone development and growth. This receptor function is regulated by Ser and Thr phosphorylation and recently, it has also been determined that it is prone to intracellular O-GlcNAc modification. Understanding the role of these chemical modifications in the regulation of the function of VDR is important to define how the products of VDR target genes are affected. In this work different computational tools were utilized to predict potential phosphorylation and glycosylation sites of VDR. Furthermore, the 3D structure of human VDR (hVDR) was constructed and docked with phosphate and O-GlcNAc. This study showed that VDR is able to bind to phosphate and O-GlcNAc, which may affect neighboring residues in a reciprocal manner to control the function of VDR.

PROPHECY OF RECOMBINANT CHYMOSIN IN *BACILLUS SUBTILIS* KO7 HOST

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Molecular analysis of a recombinant protein is a time consuming and effort taking process. The reduction in efforts and time consumption is done with the help of bioinformatics tools. Chymosin, an enzyme found in the stomach of ruminants is an important component for milk precipitation and is vital for cheese producing industries. In this study wild and optimize codon sequence of chymosin gene of *Bos taurus* with different promoters, signal peptides (SP) and different mutations in ribosomal binding site. Different bioinformatics tools apply for each above factor. The translation initiation rate of optimize codon sequence was predicted 7698.90au with the starting codon at position 13. The ΔG of secondary structure of optimize codon sequence of chymosin gene with pylb promoter and YwbN Tat signal peptide was predicted -395.7kcal/mol lower than the wild cymosin gene. We concluded that for the secretion of recombinant chymosin protein in *Bacillus subtilis* KO7 Pylb promoter with Tat SP YwbN with optimize sequence gene shows high efficiency.

ANTIBACTERIAL ACTIVITY OF CALLIANDRA SP. EXTRACTS AGAINST CLINICALLY ISOLATED STAPHYLOCOCCUS AUREUS STRAINS

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Rapid emergence of antibiotic resistance in bacteria has led to the search and development of new alternative therapeutic agents that could be used cost effectively in underdeveloped countries throughout the world. Several plants including shrubs and herbs have been used for medicinal purposes even at the time of Egyptian civilization

(Ebers Papyrus). Numerous compounds such as alkaloids, glycosides, flavonoids, phenolics, saponins, tannins, terpenes and anthraquinones present in the plants possess the antimicrobial capabilities against the pathogenic microorganisms. The main objective of this study is to determine the antibacterial activity of n-hexane and aqueous methanolic extracts of the plant (*Calliandra* sp.) against eight clinically isolated and biochemically characterized *S. aureus* strains (a causative agent of nosocomial infections) by agar well diffusion assay and to compare the medicinal activity of this plant with that of commercial antibiotic (Gentamicin). Aqueous methanolic extract of the plant was found to be more effective with inhibition zone of 18mm and 21.5mm respectively against two out of eight *S. aureus* strains as compared to Gentamicin. 2-fold dilution of methanolic extract of this plant was used to calculate the minimum inhibitory concentration by using the agar well diffusion assay and results showed that methanolic extract of *Calliandra* sp. has MIC of 1.563mg/ml against seven *S. aureus* strains while the MIC for the last strain is 0.781mg/ml. Hence, aqueous methanolic extract of plant (*Calliandra* sp.) has greater antibacterial potential against two clinically isolated *S. aureus* strains than commercially available antibiotic (Gentamicin). This result suggests that the active phytochemical constituents of this plant could be used as an alternative therapeutic agent to control the *S. aureus* associated illnesses.

AMELIORATIVE EFFECT OF OLIVE OIL AND POMEGRANATE ON OXYTOCIN INDUCE HEPATIC ANOMALIES IN MICE

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Oxytocin a neuropeptide hormone synthesize in hypothalamus used during parturition, birth and milk ejection but their ridiculous illegitimate use for cattle may produce anomalies. Study was conducted on forty, 3-4 months old male mice (*Mus musculus*) (30±3g). Animals were randomly divided as: Control (n=10) group, Oxytocin group: received 0.1ml/12h for 16 days, followed by without treatment for next 5 days, OT-PM and OT-OL were (0.1 ml/12h) pomegranate extract and olive for last 5 days respectively. The animals were euthanized by cervical dislocation on day 21th and ventricular blood was used for biochemical study and liver was used HE-stain. The histological section of Oxytocin group indicates the destruction of hepatocytes along with the sign of fibrosis and wide sinusoidal spaces along with accumulation of nuclei as compared to control. There were sign of lipogenesis and debris fills central vein along with dehydration in Oxytocin but Olive oil groups have more land marks of ameliorative effect and nullifying effects. The regulation of sinusoidal spaces also supports the regeneration ability as compared to Pomegranate.

COMPARATIVE EFFECT OF FRUCTO-OLIGOSACCHARIDES AND MANNO-OLIGOSACCHARIDES ON SERUM BIOCHEMICAL PARAMETERS IN HIGH-FAT FED RATS

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Prebiotic are defined as food ingredients that have beneficial affect on intestinal microflora, by promoting the activity or growth of bacterial population. Fructo-oligosaccharides (FOS) as well as mannooligosaccharide (MOS or mannan) act as pre-biotics which have positive effect on health of livestock and rodents. Although there is extensive research available on the microflora potential to maintain the body weight and reduction in obesity but the exact mechanism responsible for this change is still unknown. So, we aimed to sort out the mechanism responsible for regulation of metabolic activity in rats. Total twenty (N=20) male Wistar rats were in animal shed in department of Physiology, University of Animal and Veterinary Sciences Lahore. They were provided free access to water and food. Rats were distributed into four groups (n=5), Negative Control group fed standard rat chow, HFD group fed high-fat diet, MOS group fed high-fat diet supplemented with 10% MOS, FOS group fed high-fat diet supplemented

with 10% FOS. Trail was comprised of 8 weeks after 1 week of acclimatization rats in all the groups. Body weight will be recorded weekly. At the end of 8 weeks of the experiment, the blood will be collected to measure biochemical parameters, i.e. Serum glucose, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Bilirubin, total cholesterol, Triglycerides, low density lipoprotein-cholesterol (LDL-C), High density lipoprotein-cholesterol (HDL-C) with the help of commercially available kits. Data (Mean \pm SEM) were analyzed using one-way Analysis of Variance (ANOVA). Significance level among group was compared by Duncan Multiple range test in SPSS (Version 20.0). The body weight, total cholesterol and triglyceride were found no significantly change in the prebiotic supplemented group. In serum glucose, the FOS group (105.80 \pm 15.51) has comparatively better effects than MOS group (170.15 \pm 32.66) for maintaining the low level of glucose (54.66 \pm 15.67) due HFD diet to their normal level like in negative control (103.64 \pm 23.01). In case of Bilirubin, and LDL-C, MOS (0.49 \pm 0.03, 31.88 \pm 3.02) has better effects than FOS (0.57 \pm 0.05, 34.56 \pm 2.31) but not significant. While in case of AST and ALT the MOS group (27.00 \pm 2.60, 31.67 \pm 3.09) is significantly better results ($P < 0.05$) than FOS (45.14 \pm 2.66, 50.25 \pm 5.46). Our results conclude that MOS is more beneficial in term of liver activity and good cholesterol maintenance than FOS and can be recommended along the high fat diet or obese rats, but it is not good in hyperglycemic patients.

ISOLATION AND CHARACTERIZATION OF CILIATES (*PARAMECIUM* SP.) AND THEIR ROLE IN BIOREMEDIATION OF HEAVY METALS

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Utilization of toxic chemicals and heavy metals in different processes that are practiced in industrialization produces wastes that are released into water bodies. These wastes find their way in irrigations and also devastate the small microscopic creatures and other aquatic life as well. Bioremediation is a very useful way to remove toxic metals from the water sources with the expenditure of a little energy. The present study is also an abominable effort in this regard. A ciliated microscopic creature *Paramecium* sp. was isolated from stagnant water samples obtained from industrial effluents located in Faisalabad, Sheikhupura and Lahore. Five samples were purified and named as AS-1, AS-2, AS-3, AS-4 and AS-5. They showed maximum growth of 3400/ml cells at temperatures between 25°C and 30°C and pH 7.5. AS-2 was identified as *Paramecium jenningsi* by using 18S rRNA primers and processed for further studies. It was stressed with different concentrations of copper and cadmium such as 10mM, 20mM, 30mM, 40mM and 50mM and minimum inhibitory concentration was checked. Resistance against cadmium especially at its lower concentrations of 10mM and 20mM was observed where maximum numbers of cells were counted as 7200/ml and 7400/ml respectively. On the other hand in case of copper the best resistance was observed at 30mM and 40mM where maximum numbers of cells were about 2200/ml and 2000/ml respectively. Metal uptake was measured by Atomic absorption spectrophotometry, which showed maximum absorption as 2.19 μ g/ml for copper and 2.99 μ g/ml for cadmium after 96 and 72 hours of incubation respectively.

MOLECULAR CHARACTERIZATION OF METAL RESISTANT *PARAMECIUM* SP. AND ITS ROLE IN BIOREMEDIATION

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Hazardous chemicals are polluting our atmosphere coming from different sources like factories, pharmaceutical companies and vehicles etc. The ultimate recipient of all types of wastes is aquatic ecosystem. Instead of chemical remediation, microorganisms are being used to remove pollutants from water. Samples were collected from Pindi das road, Bota park, Nizam pura and Railway track of Lahore, Punjab, Pakistan. *Paramecium* sp. was isolated and characterized with the help of 18S rRNA primers. The optimum temperature and pH were found to be 30°C and 7pH. Growth patterns of *Paramecium* sp. were observed with and without metal. The minimum inhibitory concentration

of cadmium and copper was 30 μ M. Growth was observed at different concentration of copper at 10mM, 20mM, 30mM, 40mM, and 50mM. Maximum growth pattern was seen at 20mM with 4600 cells/ml on 5th day. Growth rate was minimum at 30mM with 166 cells/ml at 6th day of metal inoculation. Different concentration of Cadmium i.e. 10mM, 20mM, 30mM, 40mM, and 50mM was added to sample. At 30mM, maximum growth was observed with 2346 cells/ml on the day 10, while at day 2, minimum growth rate was observed at 50mM as 300cells/ml. Maximum metal uptake measured by atomic absorption spectrophotometry for copper and cadmium was 1.16 μ g/ml and 1.91 μ g/ml respectively.

HYPERCHOLESTEROLEMIC TREATMENT OF WHITE RABBITS

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Hypercholesterolaemia is basically excess level of cholesterol in the bloodstream. Elevated cholesterol levels are one of the risk factors for heart disease, stroke, and peripheral artery disease. Present study deals with the effect of persimmon pulp powder supplemented diet on hypercholesterolemic rabbits. For this hypercholesterolemic condition was forcefully developed in rabbits under controlled conditions. Pulp of persimmons was dried separately in hot air oven and lyophilizer. The dried pulp was grinded into powder form for further use to check its nutraceutical value. Persimmon pulp powder was used in different concentrations (10% and 20%) along with control in diets fed to rabbits for duration of four weeks. Results indicated that lowest body weight was found in group of rabbits fed on diet supplemented with 20% pulp powder. The cholesterol level was decline from 87.33 mg/dl to 76.38 mg/dl. Similarly triglyceride level was also reduced in hypercholesterolemic rabbits ranged from 108.00mg/dl to 72.00mg/dl. Likewise same trend of reduction was observed in case of LDL 9.33 mg/dl to 7.8 mg/dl whereas HDL was increased from 6.9 mg/dl to 15.7 mg/dl.

ROLE OF MULTIPLE HEAVY METAL-RESISTANT AND HEXAVALENT CHROMIUM-REDUCING *MICROBACTERIUM TESTACEUM* B-HS2 IN DECONTAMINATION OF TANNERY EFFLUENTS

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A Cr⁶⁺ resistant *Microbacterium testaceum* B-HS2, was isolated indigenously from tannery wastewater, showed maximum growth at 37°C and pH 7. *M. testaceum* B-HS2 could resist to Cr⁶⁺ (48 mM) and heavy metals upto 2 mM (As²⁺, Zn²⁺, Cu²⁺), 7 mM (Pb²⁺) and 1 mM (Cd²⁺, Ni²⁺). Maximum activity of chromate reductase enzyme was achieved at 40°C at pH 7 and was inhibited in presence of all the heavy metals tested. *M. testaceum* B-HS2 biosorption efficiency (q) for Cr⁶⁺ was 31, 38, 66 and 47 mM/g after 2, 4, 6 and 8 days, respectively. Electron micrographs confirmed further surface adsorption and increased cell size due to intracellular Cr⁶⁺ accumulation. FTIR analysis revealed the active participation of amide and carbonyl moieties in Cr⁶⁺ adsorption confirmed by EDX and SEM analysis. Cr⁶⁺ presence triggers significant production of antioxidant enzymes (APOX, SOD, POX, GST, and CAT). Moreover, elevated levels of glutathione and other non-protein thiols substantially neutralize Cr⁶⁺ generated oxidative stress. Pilot scale study revealed that *M. testaceum* B-HS2 was helpful in removing up to 96% Cr⁶⁺ from tannery effluent within 6 days and this microbial purified water is safe for the plant growth. Multiple metal tolerance and high Cr⁶⁺ reduction potential make *M. testaceum* B-SH2 an impending foundation for green chemistry to exterminate environmental Cr⁶⁺.

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

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The current research was aimed to assess the effectiveness of seafood waste biomaterial (crab shell) as a low cost adsorbent for lead (Pb) removal from aqueous solution. The Portunid crab; *Charybdis feriata*, commonly known as the orange crab was selected as biosorbent and collected from the fishery catch from the fish harbor. The raw crab shell was processed by acid and alkali solution to remove minerals and protein contents. The effects of operating parameters, viz., contact time, initial concentration and pH on Pb removal efficiency were studied. The highest removal efficiency of Pb (II) was determined at 160 min onto the biosorbent. The highest removal efficiency (98%) was observed at 25ppm Pb solution. The adsorption of metals, highly dependent on pH of the solution and the highest removal efficiency (98.5%) of Pb was observed at pH 9.0. The data for adsorption process satisfactorily fitted to the Langmuir model than Freundlich model. The interactions between crab chitin and Pb (II) ions were investigated by qualitative analysis methods (FTIR and SEM-EDS). The study explores the potential of crab shell as the seafood waste material for the eco-friendly existence of the lead contaminated waste water.

MOLECULAR CHARACTERIZATION OF MERCURY RESISTANT AND INDOLE-3-ACETIC ACID PRODUCING BACTERIA FOR THE BIOREMEDIATION OF MERCURY

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Mercury contamination is a worldwide problem due to its availability and persistence in the environment for longer period of time along with its translocation into the food chain. In the present study, a total of 60 nitrogen-fixing (NF) and indole-3-acetic acid (IAA) producing bacterial isolates were isolated from different Hg-contaminated agricultural and industrial soil and wastewater and root nodules of different leguminous plants. The potential of selected bacterial strains to remediate mercury was determined by both qualitative and quantitative assays. The results revealed that selected bacterial isolates were able to detoxify mercury up to 94% and highly Hg-resistant strains were able to grow at 50 µg/ml concentration of HgCl₂. The ability of selected bacterial strains to use as plant growth promoters, was determined by quantifying the production of IAA. The estimation of IAA production by selected bacterial strains was performed by colorimetric method. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) techniques confirmed the production of IAA ranging from 5.5 to 9.6 µg/ml. Molecular characterization of mercury resistant and IAA producing bacteria was done by 16S rDNA ribotyping. It is concluded from this study that selected bacterial strains could be used to bioremediate mercury (Hg⁺²) as well as to enhance plant growth.

OPTIMIZATION OF RECOMBINANT EXPRESSION FOR *IN VIVO* TRANSFIGURATION OF INSOLUBLE TO SOLUBLE PROTEIN

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Methionine aminopeptidase catalyzes the removal of N-terminal methionine residue from the polypeptide chain during protein synthesis. We have cloned and expressed the methionine aminopeptidase

from *Escherichia coli*. However, the recombinant protein was produced in insoluble and inactive form. Here we report the expression conditions to solubilize recombinant protein. When the gene was expressed by the induction with isopropyl β -D-1-thiogalactopyranoside or lactose equivalent expression was observed. However, the recombinant protein was produced in the insoluble form and inactive form. When the host cells were induced with lower concentration of the inducer and incubated at a lower temperature of 17 °C, the recombinant methionine aminopeptidase was produced in soluble and active form. The recombinant protein was purified to apparent homogeneity by ion exchange chromatography. Molecular mass determination by matrix assisted laser desorption/ionization-time of flight mass spectrometric analysis demonstrated that the N-terminal methionine of the recombinant protein was removed. The conditions described here can be employed to produce recombinant proteins in soluble form.

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF PULLULANASE ENZYME FROM *BACILLUS THURINGIENSIS*

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Pullulanase is an important debranching enzyme that hydrolyzes alpha (1-6) glucosidic linkage present in starch. It is used in many industries including starch and sugar industries. Production of pullulanase enzyme has been reported from different bacterial strains, but there is no report on production of pullulanase enzyme from *Bacillus thuringiensis* even yet. The objective of this study was the production of pullulanase enzyme from soil bacterium *B. thuringiensis* its partial characterization. Culture condition for production of pullulanase enzyme from *B. thuringiensis* were optimized to get the maximum production of enzyme Maximum yield of pullulanase enzyme was obtained at pH 6, at 50°C after 24h of incubation in 3% (inoculum) in the presence of a medium containing tryptone as carbon and energy source. Various parameters (pH, temperature, time of incubation, substrate concentration, substrate specificity) were also optimized to get the optimum activity of pullulanase enzyme. Maximum enzyme activity (6.33733U/ml) was obtained at pH 7, at 50°C for 20min in 4% substrate concentration in the presence of pullulan substrate as organic source. Pullulanase enzyme was partially purified by ammonium sulphate precipitation. 145kD was calculated by SDS-PAGE analysis. The results obtained in this study are useful for large scale production of pullulanase enzyme for use in industrial applications.

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF PROTEASE ENZYME FROM A NEWLY ISOLATED STRAIN OF *BACILLUS SUBTILIS* FROM SOIL

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The objective of this study was the production of protease enzyme from bacterium *Bacillus subtilis* which was isolated from soil, its partial purification and characterization. Culture condition for production of protease enzyme from *B. subtilis* were optimized to get the maximum production of enzyme Maximum yield of protease enzyme was obtained at pH 6, at 50°C after 24h of incubation in 3% (inoculum) in the presence of a medium containing tryptone as carbon and energy source. Various parameters (pH, temperature, time of incubation, substrate concentration, substrate specificity) were also optimized to get the optimum activity of protease enzyme. Maximum enzyme activity (18.258U/ml) was obtained at pH 7, at 50°C for 20min in 4% substrate concentration in the presence of casein substrate as organic source. Protease enzyme was partially purified by ammonium sulphate precipitation. 145kD was calculated by SDS-PAGE analysis. The results obtained in this study are useful for large scale production of protease enzyme for use in industrial applications.

SPINOSAD INDUCED ALTERATION IN BIOCHEMICAL PARAMETERS OF *TROGODERMA GRANARIUM*

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Wheat (*Triticum aestivum* L.) is one of the most chief cereals that are having the ability to provide 20% of the calories in the human foods. *Trogoderma granarium* is one of the most harmful pests of wheat. Development of insecticidal resistance and its ability to withstand starvation for longer time makes it one of the most destructive pests for stored products. It has been recognized as A₂ quarantine organism for EPPO and as one of the 100 worst invasive species worldwide. It can cause up to 30% postharvest losses. Spinosad is belonging to Naturalyte class of pesticide; it was commercialized by Dow Agro-sciences Ltd. It will use as major tools for the control of various pests. The current study was aimed to determine the sublethal dose of Spinosad and its toxic effect on the biochemical profile of 4th instar larvae of *T. granarium*. The sub lethal concentration LC₅₀ of spinosad was 59.51ppm against 4th instar larvae. The concentration of total protein, free amino acids and glucose was significantly increased (71.92, 0.87 and 117.5%) respectively after exposure to sub lethal dose of spinosad. The concentrations of soluble proteins, total lipids, glycogen and trehalose contents were significantly decreased (9.9, 42.1, 6.08 and 26.6%) respectively. Various detoxifying enzymes activities were also determined after exposure. The activities of catalase, amylase, invertase, trehalase, acid phosphatase and alkaline phosphatase were significantly decreased (98.5, 76.9, 54.9, 26.1, 78.1, and 69.1%) whereas cholinesterase activity was significantly increased (23.9%) after exposure to sub lethal dose of spinosad.

IMPACT OF NEONICOTINOIDS ON BIOCHEMICAL PROFILE OF *TROGODERMA GRANARIUM*

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Wheat is second highest staple food worldwide so it is essential to increase its production and supply to meet the increasing demand in future. *Trogoderma granarium* is one of the most destructive pests of wheat crop that causes great economical lose during wheat production and post harvest storage in grain commodities. Stored grains face huge loss from 33% to 73% in one season by *T. granarium*. They voraciously feed and heat the grains and have the ability to survive in starvation up to 3 years that make it more destructive for a long duration. It reduces the quality as well as seed germination of grains. *T. granarium* larvae start ingestion of embryo till seed level, leaving behind only cast. The current study was aimed to determine the toxic effect of sub-lethal dose of imidacloprid, on biochemical profile of 4th instar larvae of *T. granarium*. Imidacloprid belongs to a class of neonicotinyl insecticides and has become one of the most efficient and successfully used insecticides against chewing and sucking pests. Neonicotinoids are agonistic neuro-active insecticides, act mainly on nicotinic acetylcholine receptors on the post-synaptic membrane, targeting the species by impairment of normal nerve impulses. The sub lethal concentration LC₅₀ was 6.13ppm against 4th instar larvae of *T. granarium*. The concentration of total protein, soluble protein and free amino acid were significantly increased (5.28, 68.99 and 76.99%) respectively after treatment with LC₂₀ of imidacloprid. The concentrations of total lipid, glucose, glycogen and trehalose contents were significantly decreased (62.25, 80, 57.04 and 77.5%) respectively. Various detoxifying enzymes activities were also determined. The activities of catalase, cholinesterase, amylase, invertase, trehalase, alkaline phosphates and acid phosphates were significantly decreased (95.9, 11.73, 77.02, 40.34, 18.84, 39.57, 76.75%) after exposure to sub-lethal dose of imidacloprid.

LEAD FINDINGS FROM MEDICINAL PLANTS FOR ARTHRITIS BY IN-SILICO METHODS**Tanwir Ahmed Abbas Khichi¹, Haleema Sadia² and Shahid Raza³**¹*Department of Zoology, Government Postgraduate College, Gojrah, Pakistan*²*Department of Biotechnology, BUITEMS, Quetta, Pakistan*³*Department of Food Science and Technology, University of Central Punjab, Lahore, Pakistan*

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From ancient times, plants have been used as an important source of medicine due to the presence of a wide range of biologically active compounds. Medicinal plants are viable source of lead compounds which can be used in drug development. Lead compounds possess favorite pharmacological properties and play a key role in drug design and development. In this study, phytochemicals from two medicinal plants *Berberis lyceum* Royle and *Artemisia annua*, were selected and investigated for their anti-inflammatory properties. These plants are traditionally claimed for treating arthritis which is an inflammatory disease. This research investigation involves the search of pharmacokinetic, drug-likeness, toxicity and bioactivity profile of selected compounds on the basis of several physio-chemical parameters by computational methods to select the best lead compounds for drug development. Ten phytochemicals from each plant was selected and examined for their molecular properties and drug likeness on the basis of Lipinski's rule of five through molinspiration software. Identification of lead compound involves assessment of absorption, distribution, metabolism, excretion, toxicity (ADMET properties) earlier in the drug discovery process. In-silico ADMET analysis was done to predict the ADMET profile of the selected compounds through admetSAR. Current study indicated that two compounds out of 10 from *Berberis lyceum* Royle, (Berberine and Palmitine) and two compound from *Artemisia annua*, (Artemisinin and Camphene), showed significant bioactivity and drug-likeness score on comparison with other compounds and meet the criteria of drug-likeness. Artemisinin, Camphene, Berberine and Palmitine also qualified ADMET criteria and drug rule of five. These compounds could be used as lead for a developing effective medicine for the treatment of Rheumatoid arthritis.

EVALUATION OF KO7 (SEVEN PROTEASE DEFICIENT) ENGINEERED STRAIN OF *BACILLUS SUBTILIS* FOR PRODUCTION OF RECOMBINANT HUMAN INTERFERON ALPHA 2B (RHIFNA-2B)**Saira Ashfaq, Faiza Saleem*, Bushra Khubaib, Afshan Kaleem, Roheena Abdullah,****Mehwish Iqtedar and Shagufta Naz***Department of Biotechnology, Lahore College for Women University, Lahore*

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From ancient times, plants have been used as an important source of medicine due to the presence of a wide range of biologically active compounds. Medicinal plants are viable source of lead compounds which can be used in drug development. Lead compounds possess favorite pharmacological properties and play a key role in drug design and development. In this study, phytochemicals from two medicinal plants *Berberis lyceum* Royle and *Artemisia annua*, were selected and investigated for their anti-inflammatory properties. These plants are traditionally claimed for treating arthritis which is an inflammatory disease. This research investigation involves the search of pharmacokinetic, drug-likeness, toxicity and bioactivity profile of selected compounds on the basis of several physio-chemical parameters by computational methods to select the best lead compounds for drug development. Ten phytochemicals from each plant was selected and examined for their molecular properties and drug likeness on the basis of Lipinski's rule of five through molinspiration software. Identification of lead compound involves assessment of absorption, distribution, metabolism, excretion, toxicity (ADMET properties) earlier in the drug discovery process. In-silico ADMET analysis was done to predict the ADMET profile of the selected compounds through admetSAR. Current study indicated that two compounds out of 10 from *Berberis lyceum* Royle, (Berberine and Palmitine) and two compound from *Artemisia annua*, (Artemisinin and Camphene), showed significant bioactivity and drug-likeness score on comparison with other compounds and meet the criteria of drug-likeness. Artemisinin, Camphene, Berberine and Palmitine also qualified ADMET criteria and drug rule of five. These compounds could be used as lead for a developing effective medicine for the treatment of Rheumatoid arthritis.

IDENTIFICATION OF POTENTIAL DRUG TARGETS ASSOCIATED WITH PROTEIN KINASES

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Protein kinases are specialized enzymes, involved in phosphorylation of proteins leading to a functional change in the target protein. This change may cause some alteration in its confirmation or a change in the enzyme activity, its cellular location or association. Some class of protein kinases act as key regulators of cell function that constitute one of the largest and most functionally diverse gene families. However, the diverse and essential function mediated by kinases shows the conservation of almost 50 distinct kinase families between different organisms including yeast, mammalian kinomes and some invertebrates. Moreover, the emergence of various important protein kinases with their crystal structures gives a deep insight to understand the catalysis and regulation of different protein kinases. This study highlights the deep insights of functional importance of protein kinases, their potential targets, inhibitors, advantages and some applications extended towards future applications. The newer research technologies have made possible advancement to explore clinically and functionally important PKs.

AUTOFLUORESCENCE AND ANTICANCER ACTIVITY OF RHIZOME EXTRACTS OF *BISTORTA AMPLEXICAULIS*

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Bistorta amplexicaulis is an important medicinal herb, native to northern areas of Pakistan (Ayubia, Nathia Galli, Abbotabad etc). Plant is rich in phenolics and flavonoids contributing to antioxidant, antimicrobial and anticancer profiles. Ubiquitous presence of pyridine and flavin (ringed structures) compounds inside the living systems allows some amount of fluorescence as a fundamental attribute. Plant cells containing secondary metabolites such as flavonoids, phenolics and alkaloids possess fluorescence to some extent. Few plant based fluorophores radiate visible light such as phenolic compounds, alkaloids, terpenoids, and porphyrins containing ringed structures. Five different Rhizome extracts were prepared e.g. ethanol (REE), methanol (REM), 80% ethanol (80RE), 80% methanol (80RM) and acetone (RAC) prepared. Ultra performance liquid chromatography was used for identification of medicinally important compounds from ethanolic extract of Rhizome and Gallic acid, caffeic acid, chlorogenic acid and catechin were identified. Above mentioned extracts were also examined under UPLC to check altered profile of UPLC chromatogram. All the four extracts were tested for fluorescence using fluorimetry. The compounds present in the Rhizome extract showed excitation at 394 and emission at 421nm. Highest intensity of fluorescence was observed in ethanolic extracts. This validates the presences of alkaloids and flavonoids in the extracts. Extracts were further evaluated for their antiproliferative potentials against HepG2 (hepatocellular carcinoma) cells. The highest activity was observed in the case of ethanolic extract of rhizome i.e IC₅₀ 0.031µg/mL. This study warrants further research upon the fluorescence properties of the extract so that it may be used as autofluorescent in further cell based assays. The possible applications of plant based fluorophores are location and detection of extract during in vivo and in vitro studies without the use of staining during confocal microscopy.

SUBMERGED CULTIVATION OF MEDICINAL MUSHROOM IN HYDROLYZATE OF LIGNO-CELLULOSIC MATERIAL

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Ganoderma lucidum belongs to the family Ganodermataceae and found in Japan, China and some other parts of Asia. Traditionally it is used against diabetic, cancer prevention agent, antitumor, immunomodulation, antimicrobial and antiviral activities. Due to difficulty in field cultivation submerged fermentation was employed as a promising method for efficient and large-scale production of mycelia biomass and bioactive metabolites. Cellulose was used in the form of lignocellulosic substrate. The *Ganoderma lucidum* which is medicinal and edible mushrooms was successfully grown in the form of mycelial biomass in static submerged culture in petri plates and flasks. The present study is based on the utilization of hydrolyzates of lignocellulosic materials such as Peanut cort, Sugarcane bagasse and Wheat Straw were used after hydrolysis. A Static Fermentation Technique was employed to investigate the mycelial growth, instead of Fruiting Body. *Ganoderma lucidum* was kept up on PDA (potato dextrose agar) medium in petri dishes at 4°C and brooded at 25 °C for 5 days for the development of *G. lucidum* and generation of Ganoderic Acid. Morphology of *G. lucidum* on various Hydrolysates was white and delicate like cotton unpredictable shape, Cloud like appearance spread in general plate and multiple little sporadic white cotton like shape with string like projections. We got a Ganoderic Acid from the Hydrolysates of Peanut cort concentrate, Sugarcane bagasse concentrate and Wheat straw concentrate at a concentration of 0.006g/L, 0.011g/L, and 0.017g/L respectively.

BIOINFORMATICS BASED ANNOTATION OF HYPOTHETICAL PROTEINS: PRIORITIZING NOVEL DRUG TARGETS FOR IN VITRO STUDY

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The uncharacterized proteins that are gene-encoded products and their biological activities are not proved by experimental evidence and known as hypothetical proteins (HPs). These proteins are important due to their excessive involvement in different cellular and signaling pathways. Here, we discussed all possibilities of in-silico analysis tools and other recently reported methods for hypothetical protein characterization and biomedical applications, including drug and vaccine development. Different methodologies, including metaproteomic have been used to study protein expression by identification of HPs and comparative genomics have also come under observation due to the emergence of evolutionary study among different organisms. Structural characterization of proteins acts as a base for their functional prediction, novel drug target identification for disease treatment, vaccine production and sero-diagnosis. HPs have played major roles in different vital phenomenon for life including host adaptation, wound healing and chemotaxis. In the current era of drug and antibiotic resistance, HPs can be novel targets to treat related diseases. Identification and characterization of most HPs are under observation and will be the most promising genomic and bioinformatics techniques in structure-based drug designing and vaccine production in future.

THE COMPARATIVE PHARMACO-HISTOKINETICS OF ASPIRIN, KETOROLAC, NALBUHINE, PIROXICAM AND FLURBIPROFEN IN AVIFAUNA

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Present work was aimed at analyzing the pharmacokinetics of five different NSAIDs in domestic chicken. A total of 120 birds were used in the experiment in 2 trials viz. autumn and spring with each trial of 10 and 20 day short term experiment. Each study group was consisted of control birds (n=10) and experimental birds (n=50). Five groups of birds were treated with therapeutic dose of NSAIDs viz. Piroxicam 2mg/kg, Flubiprofen 2mg/kg, Ketorolac 3mg/kg, Nalbuphine 3mg/kg and Aspirin 12mg/kg to each bird per day. The clinical signs include depression, somnolence and variations in body temperature were observed in all experimental birds. In autumn a substantial amount of swelling was also observed on body. A decrease in body weight was observed in Flubiprofen group. All the birds showed clear signs of enteritis which indicate that these NSAIDs mainly affect birds intestine. Few apparent differences were also observed in histological analyses of heart and skeletal muscles in some treated groups. Kidney, liver and intestine showed major histopathological changes among all treated groups.

IMMUNOMODULATORY ACTIVITY OF *ARTEMISIA BREVIFOLIA* EXTRACT AGAINST MIXED *EIMERIA* INFECTION IN CHICKENS

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This study was designed to assess the immunomodulatory activity of aqueous methanolic extract (AME) of *Artemisia brevifolia* (Leaves) against mixed *Eimeria* species infection in broiler chickens. 175 day old broiler chicks were selected for this study and equally divided in five groups (A, B, C, D and E). Each group contains 35 chicks. Extract of selected plant was given orally with dose rate of (100, 200 and 300 mg/kg of body weight respectively) at 7th, 8th and 9th day of age. Group D served as positive control (Vitamin E treated) and Group E served as negative control (PBS treated). *Eimeria* infection was given to the birds at the age of 14th day. Cell mediated immunity was evaluated by 4 tests (Phytohemagglutinin-P, Concanavalin-A, Carbon clearance assay and Dinitrochlorobenzene). Humoral immunity was evaluated by microplate hemagglutination test using sheep red blood cells. Results showed dose dependent immune response of *A. brevifolia* ABE in treated groups. *A. brevifolia* ABE @ 300 mg/kg of body weight shown best results which was almost similar ($P>0.05$) to positive control group (Vitamin E treated) but significantly higher ($P<0.05$) negative control (PBS treated).

CANNABIS INDICA: AS ANTI-CANCEROUS AND GANGRENE SUPPRESSOR

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Cannabis indica, an extremely defamed and ill-reputed plant but its homeopathic tincture and seed oil possesses marvelous qualities in life-threatening ailments. We have attained successes in controlling malignant cancerous

tumour through internal use of its seed oil along with some homeopathic medicines. Local use of tincture of *C. indica* and some helping homeopathic medicines helped us in combating skin Gangrene spreading even after five surgeries. While its antiseptic effectiveness is far better than commonly used Povidone-iodine (pyodine) moreover the efficacy of its tincture in drop-doses as antibiotic is praiseworthy. As analgesic, in extremely minor quantities, its efficacy is of no match. *Cannabis indica* as anti-cancerous: A man of about 50 year old got Malignant-tumour in the urinary bladder. He decided to use our medicines. He was given *Cannabis indica* oil along with few homeopathic medicines. Soon he observed relief in his agony. The treatment was given for about one year until he got completely free from the problem. *Cannabis indica* a gangrene suppressor, antiseptic and analgesic: Lady of about 24 years got a gangrenous boil. She was treated with numerous antibiotics but it proliferated. Two skin surgeries alongwith fourth generation intra-veinous antibiotics gave no relief. The surgeon was changed he made three more surgeries and antibiotics but failed. We used *C. indica* tincture along with some homeopathic medicines. Gangrene came under control. It means that the action of *Clostridium perfringens* was checked by *C. indica*. The same kind of extract was used against Gangrene of leg of another lady of 80 year the result was magical, she was saved from amputation. As Analgesic the tincture is given in One or Two drops and local use of it gives miraculous relief.

TOXICOLOGICAL EFFECTS OF METALS WITH DIFFERENT CONCENTRATIONS AND TYPES ON THE BIOCHEMICAL PERFORMANCE OF SHELLFISH SPECIES

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Seafood is a very common source of aquatic toxicant exposure to humans. An unknown number of naturally occurring contaminants find their way into seafood through water. On the other hand, more than 2500 chemical substances such as heavy metals are added to foods to modify or impart flavor, color, stability, and texture to fortify or enhance the nutritive values or to reduce the cost. Large amounts of heavy metals end up in the aquatic environment as a result of ever-increasing anthropogenic activities and economic development. This study examined the biochemical performance in three shellfish species on different concentrations of three heavy metals includes; Cadmium (Cd), lead (Pb) and Zinc (Zn). The protein, carbohydrate, lipid, moisture and metallothionein content in shellfish species significantly varies in between (either decreased and increased) when treated with three types of heavy metals. The study revealed that the three types of heavy metals significantly affect the biochemical performance of shellfish species; the toxicological effects of heavy metal increase in the biochemical performance of all treated shellfish with the increase of heavy metal concentrations.

EFFECT OF DRYING ON ETHER EXTRACT AND PROTEIN CONTENTS OF ROHU (*LABEO ROHITA* AND MORI (*CIRRHINUS MRIGALA*))

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Fish is more susceptible to deterioration due to microbial activity, so it requires appropriate methods to preserve it so that its shelf life could be enhanced. For establishment of effective technique for preservation, awareness of processes ultimately leading to spoilage is necessary. In present study, ether extract, moisture, protein contents and dry matter contents of fresh and sun dried fish samples of Rohu (*Labeo rohita*) and Mori (*Cirrhinus mrigala*) were analyzed. Results revealed that fresh samples of Rohu (*Labeo rohita*) had higher value of moisture content (75.60 ± 1.84) as well as fresh samples of Mori (*Cirrhinus mrigala*) also had greater moisture content in it (76.47 ± 0.72). Apart from greater moisture content, fresh samples of Rohu (*Labeo rohita*) had lower values of ether extract (7.42 ± 1.51), protein contents (8.87 ± 4.75) and dry matter content (3.80 ± 0.35). Similarly, lower values of ether extract (9.50 ± 0.37), protein (9.58 ± 5.32) and dry matter content (4.27 ± 0.90) were recorded in fresh samples of Mori (*Cirrhinus mrigala*). Sun dried samples of Rohu (*Labeo rohita*) had lower moisture content (14.71 ± 0.69), greater protein content (20.02 ± 1.73), higher ether extract (16.96 ± 0.43) and increased value of dry matter (19.85 ± 0.58).

Likewise, sun dried samples of Mori (*Cirrhinus mrigala*) had lower moisture content (15.20 ± 0.62), higher protein content (21.70 ± 1.86), greater ether extract (21.52 ± 0.40) and increased value of dry matter (21.05 ± 2.13). Regression analysis and one way analysis of variance showed that sun drying had significant effect on ether extract, protein, dry matter and moisture of both fish species mentioned. From present study it was concluded that sun dried fish is more preferable and nutritious because of high protein content and low moisture pointed towards extended and longer shelf life.

PROTECTIVE ROLE OF HEMP AGAINST MERCURY INDUCED TOXICITY IN CYPRINID

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The present study was performed to assess the role of hemp in providing protection against mercury toxicity in *Cyprinus carpio*. The LC₅₀ of Mercuric Chloride (HgCl₂) for 96 h was determined. The one fifth of LC 50/96 h was used for further toxicity evaluation. A completely randomized experiment was run in triplicate. The control group G₀ was fed without hempseed, while treated groups, G₁ and G₂ were provided with 20% and 40% hempseed diet respectively. After 15 days of feeding, treated groups showed significant increase in values of red blood cells, white blood cells, hematocrit, hemoglobin, activities of acetylcholinesterase, alkaline phosphatase, hepatic total protein, and decrease in mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, activity of antioxidant enzymes, super oxide dismutase, catalase, peroxidase, lipid peroxidase, and metabolic enzymes, lactate dehydrogenase, alanine transaminase, and aspartate transaminase as compared to control group. However, after 15 days exposure to LC₅₀ of HgCl₂ to both control and hempseed fed groups, opposite trend of all above mentioned parameters was observed. Furthermore, histopathological study of liver, gills and intestine revealed disorganization, edema, hyperplasia, while hemorrhage, hydropic swelling and hemosiderosis and narcosis in liver and intestinal tissues. However, G₁ and G₂ groups showed less toxicological effects as compared to control G₀ and G_{0t} exposed to HgCl₂. It seems, hempseed improved the health status of fish and provided strength to combat toxicological effect of mercury. Thus, utilization of hemp is suggested for improving defense mechanism of fish.

EFFECT OF FREEZING ON ETHER EXTRACT AND PROTEIN CONTENT OF ROHU (*LABEO ROHITA*) AND MORI (*CIRRHINUS MRIGALA*)

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Fish is an important source of protein rich in essential amino acids and has important elements for the maintenance of healthy body. Freezing is the main method of processing fish for human consumption and the most used to reduce biochemical changes that occur during storage. Fish (42 fillets of each species) were purchased from Baigowala fish farm Sialkot. At fresh condition 6 samples were analyzed to determine fat and protein contents. The remaining samples were refrigerated at two different temperatures (0°C and -20°C). The ether extract and protein content were analyzed after each 15 days from the start of experiment. Compositional changes during fish spoilage results in lipid oxidation and protein degradation as well as the loss of other valuable molecules. Results showed that highest protein (19.68 ± 1.78 % of Mori and 21.87 ± 1.78 % of Rohu), ether extract (14.01 ± 0.46 % of Mori and 12.62 ± 5.91 % of Rohu) and dry matter content (31.55 ± 2.53 % of Mori and 25.54 ± 1.28 % of Rohu) and lowest moisture content (68.45 ± 2.53 % of Mori and 74.45 ± 1.28 % of Rohu) were found in freshly analyzed samples. Protein content (14.57 ± 3.71 % of Mori and 13.12 ± 1.78 % of Rohu), ether extract (5.87 ± 2.21 % of Mori and 6.23 ± 0.87 % of Rohu) and dry matter content (23.12 ± 1.09 % of Mori and 18.54 ± 0.78 % of Rohu) showed reducing trend, on the other hand moisture content percentage (76.88 ± 0.68 % of Mori and 81.48 ± 0.49 % of Rohu) showed increasing trend during whole storage period. Data is statistically analyzed after the completion of 45 days of experiment. Frozen storage cause reduction in protein content and ether extract in both fish species.

MANGO SEED KERNEL EXTRACT AS A NATURAL ANTIMICROBIAL AGENT**Babar Ali, Umar Farooq, Afshan Shafi and Abdul Wali Khan***Department of Food Science and Technology, MNS-University of Agriculture, Multan, Pakistan*

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Continues spread of microbial infectious diseases which affect almost 50, 000 people every day. So this alarming condition has necessitated search of new and natural antimicrobial substances with higher bioactivity and no side effects. The current research was planned to control food borne pathogens by using mango seed kernel extract as natural antimicrobial agent. The antimicrobial activity of mango kernel extracts was examined by disc diffusion assay against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacterial strains. The aqueous and ethanolic extracts of mango seed kernel were obtained at different temperatures to check their antimicrobial potential. The results directed that extracts of mango seed kernel possessed noteworthy antibacterial activity of clinical significance against all tested bacterial strains. From these results it was concluded that the food borne pathogens and diseases can be controlled by the use of mango seed kernel extracts which might be a cheaper and safe (natural) way of treatment.

ANTI-MICROBIAL EFFECT OF BANANA PEEL AGAINST FOOD BORNE PATHOGEN**Khizar Hayat, Umar Farooq, Afshan Shafi and Muhammad Ishaq***Department of Food Science and Technology, MNS- University of Agriculture, Multan*

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Food borne illness occurs because of some infectious pathogens, their action change the entire metabolism of human beings and create the adverse condition which causing numerous deaths each year. The current research has been planned to study the anti-microbial potential of banana peel aqueous and ethanolic extracts against food borne pathogens. The banana peel aqueous and ethanolic extracts were prepared in various combinations of water and ethanol. These extracts were evaluated for different phenolic components and antimicrobial potential against (food borne pathogens) *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhus* and *Escherichia coli*. The aqueous and ethanol extract of banana peel extract at different temperature and form treatments with different ratios of water and ethanol to observe the antimicrobial potential against *Bacillus subtilis*, *Salmonella typhus*, *E. coli*, *Staphylococcus aureus* through disc diffusion method. This indicates that the organic solvent (ethanol) extracts exhibited greater antimicrobial activity that might be due to non-polar behavior of most of the antimicrobial compounds in the plant sources and hence were extracted more through the organic solvent medium. It concludes that banana peel have effective medicinal property and can be used by traditional medical practitioners.

TREATMENT OF HYPERCHOLESTROLEMIC THROUGH APRICOT KERNEL OIL**Shanza Arbab, Umar Farooq and Afshan Shafi***Department of Food Science and Technology, MNS-University of Agriculture, Multan*

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The present research study was based on the assessment of therapeutic potential of diet supplemented with apricot kernel oil through biological model. In this study white Newzealand rabbits were used as experimental model. Diet formulations were of two forms one to induce hypercholesterolemia in rabbits whereas second was the treatment supplemented diet. Hypercholesterolemia was confirmed from study of blood samples and rabbits were fed with treatment diet containing apricot kernel oil for 42 days. Results of present research work the maximum decline in blood cholesterol level of rabbits after 45 days of treatment was observed in rabbits fed on diet supplemented with 1% apricot kernel oil with reduction of 155.52 mg/dl to 117.27 mg/dl. It was also observed that HDL increased from 16.85mg/dl to 21.80mg/dl and LDL was decreased from 35.19mg/dl to 27.31mg/dl. It was accomplished that apricot kernel oil possess significant antimicrobial potentials against food borne pathogens.

BIOLOGICAL CONTROL OF POTATO COMMON SCAB WITH RARE ISATROPOLONE C COMPOUND PRODUCE BY PLANT GROWTH PROMOTING *STREPTOMYCES* A1RT

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Potato is prone to many drastic diseases like potato common scab (CS). As no highly effective methods exist for managing CS, this study explored the possibility of using biological control. Ten bacterial strains were isolated from CS-infected potato tubers from four different locations of Punjab, Pakistan, and identified based on biochemical and molecular analysis. Analysis of 16S rDNA sequences amplified by PCR revealed the isolated bacterial strains to be *Streptomyces scabies*, *S. turgidiscabies* and *S. stelliscabiei*. Pathogenic islands were also confirmed among the isolates after identification of *txtAB*, *nec1*, and *tomA* genes with PCR amplification. One strain isolated from soil was antagonistic to the pathogenic *Streptomyces* spp., and determined to be *Streptomyces* A1RT on the basis of 16S rRNA sequencing. A methanolic extract of *Streptomyces* A1RT contained Isatropolone C, which was purified and structurally determined by ¹H- and ¹³C-NMR, ¹H/¹H-COSY, HMQC, and HMBC techniques. *Streptomyces* A1RT also produced the plant growth hormone indole-3-acetic acid (IAA) with a titer of 26 mg ml⁻¹ as confirmed by spectrophotometry and HPLC. In a greenhouse assay, disease severity index was established from 0 to 500. Average disease severity indexes were recorded as 63, 130.5, and 78 for *Streptomyces scabies*, *S. turgidiscabies* and *S. stelliscabiei*, respectively. When *Streptomyces* A1RT was applied in soil that contained one of these pathogenic isolates, the average disease severity indexes were significantly ($P < 0.05$) reduced to 11.1, 5.6 and 8.4, respectively. A significant increase in tuber weight and shoot development was also observed with the tubers treated with *Streptomyces* A1RT. The use of the plant growth-promoting *Streptomyces* A1RT against potato CS thus provides an alternative strategy to control the disease without affecting environmental, plants, animals and human health.

IN VITRO ANTI-INFLAMMATORY AND α -CHYMOTRYPSIN INHIBITION ACTIVITIES OF ETHANOLIC EXTRACTS OF FOUR TRADITIONAL MEDICINAL PLANT EXTRACTS

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Proteases are essential for the survival of an organism, but become nuisance when overexpressed. α -Chymotrypsin is a protease enzyme that along with cathepsin results in inflammatory arthritis. Biological components present in medicinal plants are potent inhibitors of α -chymotrypsin. The aim of current study was to find out inhibition potential of four plants against α -chymotrypsin. Ethanolic extracts of *Momordica charantia*, *Trigonella foenum-graecum*, *Aloe vera* (leaves), *Aloe vera* (gel) and *Trachyspermum ammi* were tested and their IC₅₀ values were calculated. Among these plants, *Aloe vera* (gel), *Aloe vera* (leaves) and *Trigonella foenum-graecum* showed significant inhibition activity with IC₅₀ values 23 μ g/ml, 25.9 μ g/ml and 72.8 μ g/ml respectively.

SACCHARIFICATION OF SWEET POTATO PEEL: AN ALTERNATIVE AND SUSTAINABLE SOURCE FOR THE PRODUCTION OF α -1, 4-GLUCOSIDASE FROM *BACILLUS LICHENIFORMIS* KIBGE-IB4

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The biodegradable agro-industrial wastes are mostly considered as potential sustainable source for the production of various value-added products from microbial species. Due to easy availability and economical profitability, agro-industrial wastes are preferred for large-scale production of enzymes and also to improve microbial cell growth. The hydrolytic enzymes can selectively hydrolyze the internal linkages of complex carbohydrates to release glucose moieties which can be further utilized in different industrial bioprocess. In the current study, sweet potato peel (*Ipomoea batatas*) was observed as the most favorable substrate for the maximum synthesis of α -1, 4-glucosidase among various agro-industrial wastes. *Bacillus licheniformis* KIBGE-IB4 produced maximum quantity of α -1, 4-glucosidase when growth medium was supplemented with 1% substrate. It was evident from the results that bacterial isolate secreted high titer of α -1,4-glucosidase in the presence of peptone, yeast extract and meat extract with optimum concentration of 0.4%, 0.1% and 0.4% respectively. *Bacillus licheniformis* KIBGE-IB4 revealed maximum enzyme productivity at 40°C and pH-7.0 after 48 hours of fermentation period. An improved and cost effective growth medium design resulted 570.63±28.53 U mg⁻¹ of α -1,4-glucosidase from *B. licheniformis* KIBGE-IB4. This enzyme can be used to fulfill the accelerating demand of food and pharmaceutical industries. Further purification and immobilization of this enzyme can also enhance its utility for various bioprocesses.

UTILIZATION OF FISH POND SEDIMENTS AS BIOFERTILIZER FOR THE GROWTH OF *ABELMOSCHUS ESCULENTUS* (L.) (OKRA)

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The aim of the present study is to investigate the effects of fish pond sediments utilized as bio fertilizer on productivity potential of *Abelmoschus esculentus* (L.) (Okra). The experiment was carried out at Jinnah University for Women, Karachi, Pakistan from June to November 2018. For the experiment fish pond sediment that utilized was collected from the Private Fish Farm. Collected fish pond sediment dried outside the pond then smashed it and along with ballu mitti (sand) it weighs it in 3 different amounts. There were 4 treatments of different ratios, i.e., 1:3, 1:1, 3:1 and controlled pot. The experiment was performed in pots. Rahman and Yakupitiyage in 2006, utilized tilapia fish pond sediment for the production of morning glory and described that pond sediment can intensify the nutrient level in soil, pH and nutrient availability increased. Amira and Suloma in 2013 utilized the intensive catfish, tilapia and biofloc tilapia fishes effluents as fertilizer for production of Khaya and mahogany seedlings and got the best results on these utilization. Major and minor variations were also observed in aspects of germination, quality of growth, fruits and pest activity. Experimental results revealed that the germination rate is different in different ratios of treatments.

EFFICACY AND TOXICITY OF PROFENOFOS ON THE ENERGY RESERVES AND ENZYMATIC ACTIVITIES OF *TROGODERMA GRANARIUM*

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Evaluation of Profenofos for the management of highly voracious and harmful insect pest of cereal grains, Khapra beetle (*Trogoderma granarium*) was demonstrated in this research work. For this purpose two populations of 4th instar larvae were selected, one was Susceptible population had no history of insecticide exposure while other was collected from the godown of Layyah and it previously had exposure of deltamethrin. The LC₂₀, LC₅₀ and LC₉₉ values of Profenofos against 4th instar larvae of Susceptible populations were found to be 117.68, 328.06 and 909.58ppm respectively. The LC₂₀, LC₅₀ and LC₉₉ values for 4th instar larvae of Layyah populations were found to be 193.61, 615.35 and 1781.08ppm respectively. The effect of sub-lethal dose (LC₂₀) of Profenofos was selected to study its mode of toxicity and percentage change in each parameter was corresponded to control (treated with absolute acetone) for each population. The LC₂₀ dose of Profenofos significantly increased the level of total soluble protein and trehalose contents but the level of total protein, free amino acids, glucose and glycogen contents were decreased in Susceptible population. Whereas in Layyah populations there were an inclination resulted in glucose contents but insignificantly reduction observed in contents of total soluble protein, trehalose, total protein, free amino acids and glycogen respectively. In both Susceptible and Layyah populations the detoxification enzymes such as cholinesterase, trehalase, amylase, invertase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities were dropped down. This data suggests that by getting the information about previous history of insecticide exposure for insect control their population buildup can be prevented at low dose level by using insecticide of different mode of toxicity which not only prove beneficial for controlling insects but also safe for environment and human health.

CLUSTERING OF MODIFIABLE CARDIOVASCULAR DISEASE RISK FACTORS IN ADULTS AGED <40 IN DISTRICT HYDERABAD

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Cardiovascular diseases are leading cause of death and disability around the world. A number of Studies have clearly stated that People from Indian subcontinent, including Pakistan are more at Risk for developing CVDs as compared to other countries. This modifiable cardiovascular risk factors are obesity, Hypertension, increased cholesterol levels, increased blood glucose levels, smoking, sedentary lifestyle, and unhealthy diet. A cross-sectional study was carried in 2015 to determine prevalence of modifiable risk factors in the population of Hyderabad district. Healthy adults <40 years of age from Hyderabad district were recruited. Subjects over 40 years of age and those with history of CVD, those who were on medication, pregnant women, smokers and drug addicts were excluded. A self-designed structured questionnaire was administered to all participants and associated data was collected. Anthropometric measurements and blood pressure of subjects were taken in standardized way. Blood was collected and serum was obtained by centrifuging the samples at 2000 rpm for 5minutes. Serum was than kept at -20 centigrade until analyzed. Samples were analyzed using autoanalyzer Microlab 300 by Merck. Out of total subject 76% had at least one risk factor present. Prevalence of Obesity (>25 BMI) which is the main CVD risk factor was 29% and 30% for men and women respectively. Central obesity, another measure widely used for assessment of CVD risk, was higher in female (61%) as compared to male (35%) (>80cms in women and >90cm in men). Of total, 34% of subjects had increased blood pressure (>130 systolic), being higher in male (40%) than females (22%). Cholesterol and Blood Glucose levels were increased in 7%. Triglyceride count was increased in VI 35% of total subjects. While HDL, considered as good cholesterol was less than desired in 26% subjects. Out of all risk factors, LDL, deemed as a major cause of atherosclerosis, was least prevalent (6%) of total subjects. Conclusively, this study will help in making public health policies.

2. CELL BIOLOGY AND GENETICS

MOLECULAR STUDY OF OCULOCUTANEOUS ALBINISM THROUGH LINKAGE ANALYSIS

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Albinism represents a group of genetic disorders with a broad spectrum of hypo-pigmentary phenotypes dependent on the genetic background of the patients. Foveal hypoplasia is invariably present and individuals with albinism often have delayed visual development, reduced vision, nystagmus, strabismus, iris trans-illumination, and absent or reduced melanin pigment. Oculocutaneous albinism (OCA) patients have little or no pigment in their eyes, skin and hair, whereas ocular albinism (OA) primarily presents the ocular symptoms and the skin and hair color may vary from near normal to very fair. Mutations in genes directly or indirectly regulating melanin production are responsible for different forms of albinism with overlapping clinical features. Most commonly mutations in the Tyrosinase, P gene / OCA2, Tyrosinase related protein (TYRP1), SLC45A2, SLC24A5, C10orf11, GPR143, CACNA1F causes albinism. Clinical examination of patients affected with oculocutaneous albinism was done. Samples were collected from the patients of oculocutaneous albinism. After gel electrophoresis PCR was performed. The samples were sent for the sequencing. The results were analysed. Missense mutation of pV427A was detected in exon 4 of family OCA002. The mutation in the affected family of oculocutaneous albinism in the *TYR* gene will promote awareness about the basis of oculocutaneous albinism 1A at molecular level.

GENETIC SCREENING FOR C70R HAMP GENE MUTATION AND ROLE OF IRON OVERLOAD IN HEMOCHROMATOSIS B-THALASSEMIA PATIENTS OF LAHORE

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Hereditary Hemochromatosis is a rare genetic iron overload disorder in which iron accumulates in vital body organs like lungs, liver, spleen, and pancreas due to mutations in HFE, TFR2, TFR1, HJV and HAMP genes. In HFE gene, C282Y and H63D mutations cause dys-regulation of iron in Hemochromatosis patients. Along with HFE, HAMP gene mutations are principle source of disturbance of iron homeostasis. For the first time in Lahore, C70R mutation was screened by PCR-RFLPs in exon 3 of HAMP gene in 27 controls and 106 β -thalassemia patients and sequencing of 8 HCV⁺ patients was performed due to higher level of ferritin in their blood along with secondary complications splenomegaly leading to splenectomy, hepatomegaly and ascites. Sac II restriction enzyme was used to screen genetically affected and control samples but no patient with C70R mutation was found in the population of Lahore region. Further presumption was anticipated using bioinformatics tools for structural and functional manifestation of mutated protein. Energy difference (ΔG) between mutated C70R protein and wild-type is found to be +1.93kcal which demonstrates increase in stability. Future recommendations include sequencing of complete hepcidin gene with its three exons and large sample size.

CLINICAL AND MOLECULAR CHARACTERIZATION OF CONGENITAL ALOPECIA WITH MENTAL RETARDATION SYNDROME (APMR4) IN PAKISTANI FAMILY

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Congenital alopecia with mental retardation syndrome is a rare autosomal recessive disorder characterized clinically by complete hair loss and moderate to severe mental retardation. In the present report a five-generation Pakistani consanguineous kindred with multiple affected individuals were presented with alopecia and severe mental retardation has been ascertained from a remote region in Pakistan. In 2006-2007, we have reported the mapping of three distinct loci APMR1 and APMR2 on chromosome 3q26 and APMR3 on chromosome 18q11. The clinical course for affected patients of APMR4 family is similar to APMR1 reported by our group Peter et al. (2006). Genotyping using microsatellite markers linked to the region showed linkage in this family to APMR4 loci on chromosome 3q25.1-q28. A maximum two-point LOD score of 1.74 ($\theta=0.0$) was obtained at several markers. Multipoint linkage analysis resulted in a maximum LOD score of 3.17 with markers D3S2433, D3S2427 and D3S3676 which supports the linkage. Recombination events observed in affected individuals localized the disease locus between markers D3S1299 and D3S3596, spanning 41.78-cM region on chromosome 3q25.1-q28. The linkage interval of the APMR4 locus identified here overlaps with the APMR1 and APMR2 (Figure 1). Sequence analysis of five candidate genes, *LIPH*, *ETS variant gene 5*, *TNFSF10*, *AP2M1* and *CAM-K2N2* from DNA samples of two affected and one normal individual of the family revealed no potentially causal variants. In conclusion, this study results suggest the existence of an additional gene locus for autosomal recessive APMR within one of the four linkage intervals, and they reveal that APMR is an intriguingly heterogeneous neuroectodermal genetic disorder. The APMR causative genes are possibly connected in a common pathway or protein complex, and their elucidation will provide valuable insights into the development of skin appendages and the brain.

SCREENING OF THE CANDIDATE GENE INVOLVED IN AUTOSOMAL RECESSIVE INTELLECTUAL DISABILITY AND MICROCEPHALY

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Intellectual disability affects 1–3% of the general population and the environmental or hereditary etiology is identified in less than half of patients. Genetic studies have reported mutations in approximately 1000 different genes that may cause intellectual disability. We assessed patients from two consanguineous Pakistani families from Kashmir region, exhibiting non-syndromal intellectual disability and postnatal microcephaly with whole exome sequencing (WES) followed by Sanger sequencing and cosegregation analysis. WES analysis of both families revealed pathogenic mutations in the *TRAPPC9* gene (NM_031466.7), which has been linked previously to autosomal recessive mental retardation type 13 (MRT13; MIM#613192). The novel nonsense mutation c.2065G>T (p.Glu689*), and the previously identified mutation c.1423C>T (p.Arg475*) were verified through Sanger sequencing and cosegregation analysis. Mutations in *TRAPPC9* were frequently encountered in 7 unrelated families, highlighting a possible hotspot for ID-causing mutations.

MOLECULAR ANALYSIS OF *TRMT1* GENE INVOLVED IN AUTOSOMAL RECESSIVE INTELLECTUAL DISABILITY AND MICROCEPHALY

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The *TRMT1* gene (MIM#611669) encodes the protein tRNA methyltransferase 1 that transfers a methyl-group onto a single guanine residue present in most tRNAs and thereby modifies them posttranslationally. The exact function of *TRMT1* is unknown, but such methyltransferases have been suggested to influence parameters such as RNA stability, translation, trafficking, localization, enzymatic activity or patterns of interactions with other molecules. Homozygous mutations in the *TRMT1* gene have been reported recently in a large screen for genes associated with autosomal recessive intellectual disability. So far only two families with a biallelic *TRMT1* mutation have been reported; however, no detailed clinical or radiological data was available. Here, we report four further individuals who carry homozygous mutations in the *TRMT1* gene and thereby expand the *TRMT1*-linked phenotype. We investigated affected and non-affected individuals of the two families through whole exome sequencing (WES) approach and identified a novel 1 bp insertion at the donor-site between exon 12 and 13 (c.1506+1G>T, in family 2 and an already reported homozygous deletion in the *TRMT1* gene (c.657_688del32, p.Q219Hfs* in family 1). Both reported mutations affect highly conserved amino acids within the functional domain of TRMT1.

TETRA PRIMER ANALYSIS OF FAMILIES WITH HEREDITARY OCULOCUTANEOUS ALBINISM

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Albinism derived from Latin word “Albus” meaning “White” is a group of genetic disorder of impaired biosynthesis of melanin, produces diverse phenotype that are categorized on the basis of mutation(s) on a gene that result to cause albinism. Its global prevalence that has been reported is approximately 1:17000, which means that 1 in 70 people are the carrier of OCA allele. In the present study two families affected with autosomal recessive non syndromic OCA were recruited from remote areas of Azad Jammu and Kashmir. Tetra primer analysis followed by whole exome sequencing identified a previously reported mutation c.1255G>A with protein modification p.(Gly419Arg) in one family in *TYR* gene. In second family two compound heterozygous mutations c.827 T>A p.(Val276Glu) and c.877G>C p.(Glu293Gln) are identified in *OCA2* gene. The mutations identified through whole exome sequencing were confirmed through Sanger sequencing. Hence this study broadens the spectrum of mutations in *TYR* gene in Pakistani population. In second family further research work is recommended to identify the actual causative variants. Our results correlate with the previously reported mutation in *TYR* gene. This study also shows that whole exome sequencing is a powerful and rapid technique for the identification of causative variants in various genetic disorders.

COMPARATIVE STUDY OF VARIOUS GREEN FODDER YIELD AND QUALITY CONTRIBUTING TRAITS IN SORGHUM-SUDANGRASS HYBRIDS UNDER DIFFERENT MOISTURE LEVELS

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Quality and cheap fodder availability is key factor to nourish livestock. Which is declining continuously due to abiotic stresses especially drought. Development and evaluation of high yielding multi-cut sorghum hybrids is need of hour to cope with fodder shortage condition. Therefore, 10 sorghum hybrids were planted under different soil moisture conditions at research area of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Correlation study showed a negative and non-significant association between fodder yield and quality contributing traits under different moisture levels. Where, NDF and ADF showed positive and significant correlation. Overall, all the green fodder yield contributing traits reduced under moisture stress. While, Protein contents increased under moisture stress in most of the hybrids. Among all the sorghum hybrids, A6 × SG3 and A7 × SG1 showed best performance regarding fodder yield and quality traits under moisture stress.

GENOTOXIC POTENTIAL OF PESTICIDE MIXTURE AGAINST COMMON CARP

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Contamination of water due to pesticide residues is a global problem. Pesticides come from industrial and agricultural effluents, reach in the aquatic environment where it poses significant toxicological risks to non target organisms. Pesticides finding their way to the food chain threatening the ecological balance and biodiversity of the natural ecosystems. Therefore, this study was designed to determine the genotoxic potential of two commonly used pesticides viz. chlorpyrifos and bifenthrin in the form of mixture on commercially important freshwater fish common carp (*Cyprinus carpio*). For this purpose 180 day old fish fingerlings were exposed to sub-lethal concentrations viz. 1/3rd, 1/4th, 1/5th and 1/6th of 96-hr LC₅₀ of mixture for a period of two months at constant laboratory conditions. Fish blood erythrocytes were sampled on day 30th and 60th of exposure period for the assessment of DNA damage. Genotoxic effects of mixture were determined in terms of percentage of damaged nuclei in the peripheral blood erythrocytes of fish. Statistically significant effects ($p < 0.05$) for both concentration and time of exposure were observed in treated and control fish. The level of DNA damage in terms of percentage of damaged nuclei was significantly higher on day 60th of exposure at all sub-lethal concentrations as compared to 30th day. The DNA damage was found to be dose and time dependent with highest DNA damage observed at 1/3rd of LC₅₀, followed by that of positive control, 1/4th, 1/5th and 1/6th of LC₅₀ exposures as compared to negative control. This study confirmed that the comet assay is a useful tool for assessing the genotoxic potential of waterborne pollutants and might be appropriate as a part of environmental monitoring programs.

ATP13A4 GENE INVOLVED IN EARLY INFANTILE EPILEPTIC ENCEPHALOPATHY

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Epilepsy is the condition which is marked as two or more than two unprovoked seizures within 24 hours. It is most common neurological disorder in the world. It showed disturbance in brain activity which caused seizure. It is more common in less developed countries as compared to advanced countries. Epilepsy is the major health issue

which accounts 0.7% of the total disease burden in the world. . In urban areas of Pakistan the epilepsy rate is 7.4/1000 whereas in rural areas the rate is doubled than urban areas i.e 14.8/1000. Prevalence of epilepsy in developed countries is 8.4 per 1000 people. We exclude patients which face head injuries and brain malformation. The objective of this study was to find out the genetic variants that can play significant role in causing epilepsy in Pakistan. Data was analyzed through whole exome sequencing. More than 1500 genes involved in epilepsy. Mutations in some genes caused epilepsy and seizures. Voltage gated and ligand gated ion channels play significant role in epilepsy. Dravet syndrome is more common in children. It is characterized by seizure types such as tonic clonic, febrile and afebrile. Most common genes mutation which involved in dravet syndrome are SCN1A, SCN1B, GABRG2, CHD2, PCDH19 and GABRA1. EFHC1, GPR98 and ATP13A4 play a role in juvenile myoclonic epilepsy and generalized epilepsy. Mutation in ATP13A4 is associated with early infantile epileptic encephalopathy. Pathogenic variant identified in gene ATP13A4 which is associated in early infantile epileptic encephalopathy. We studied mutation in patient of gene ATP13A4 showing miss sense variant of snip rs35424709. Amino acid change take place at position Glu646Asp and nucleotide change take place at position 1938A>T. This gene involved in early infantile epileptic encephalopathy. This study extends our comprehensive understanding of epilepsy that is associated with children in early age.

THE TRIBE AELURILLINE SPECIES FIRST CYTOGENETIC STUDIES FROM PAKISTAN

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Aelurillus logovnovi Azarkina 2004, *Stenaelurillus mardanicus* Ali and Maddison, 2018 *Langona cf. tartarica* (a complex sympatric species association) of Tribe aelurilline were subjected for the first time for karyotyping. The male XXO and female XXY were observed in all the species the 2n diploid condition are more complex to understand in all the species. The *Langona cf. tartarica* species complex is need to more understand and to be precisely separated.

MOLECULAR ANALYSIS OF NONSYNDROMIC ICHTHYOSIS IN QUETTA, PAKISTAN

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Inherited ichthyosis is a heterogeneous group of disorder of cornification, involves whole integument. This heterogeneity is due to mutations in various genes concerned in keratinocyte differentiation and epidermal barrier function. According to consensus conference held in 2009, inherited ichthyosis classified into two major classes 1) syndromic ichthyosis and 2) non syndromic ichthyosis. X-linked ichthyosis is a second most frequently occurring nonsyndromic ichthyosis. Clinically it is characterized by brown adherent scales. These scales are present on whole body except face, palms and soles. Research showed that 90% of X-linked ichthyosis cases were because of the complete deletion of *STS* gene. This gene encodes the steroid sulfatase enzyme which helps in formation of stratum corneum. SC has a vital role in maintaining skin barrier function. Autosomal recessive congenital ichthyosis ARCI comprised of three major sub types 1) harlequin ichthyosis HI, 2) lamellar ichthyosis LI, 3) congenital ichthyosiform erythroderma CIE. The phenotype of ARCI are white or gray scales on whole epidermis with erythema in some cases, collodion membrane, transepidermal water lose, hyperkeratosis, eclabium, ectropion and oftenly alopecia. The prevalence rate of ARCI is 1 in 20000 individuals and the most frequent cause is mutation in *TGM1* gene. In the current study, four families, DER 70 affected with X-linked ichthyosis. DER 69, DER 68, and DER 71 affected with ARCI were contemplated. All families were recruited from Quetta, capital of Baluchistan, Pakistan. Patient of DER 69 showed the clinical features of lamellar ichthyosis, while patients of DER 68 and DER 71 present the typical

features of CIE with erythma. Keeping in view the high incidence rate of *TGM1* mutations in ARCI patients, the families were screened for mutations in *TGM1* gene through Sanger sequencing method. No pathogenic mutation was found in *TGM1* gene. In DER 70, patient is affected with X-linked ichthyosis, the analysis of DER 70 through PCR showed the complete deletion of *STS* gene.

EXOME SEQUENCE ANALYSIS IN CONSANGUINEOUS PAKISTANI FAMILIES INHERITING BARDET BIEDLE SYNDROME DETERMINED FOUNDER EFFECT OF MUTATION C.299DEL C (P.SER100LEUFS*24) IN *BBS9* GENE

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Bardet biedl syndrome (BBS), a condition of defective ciliogenesis, is characterized by heterogeneous phenotypic spectrum that involves retinopathy, intellectual disability, obesity, polydactyly, and kidney dysfunctions as the major clinical feature. Genetic investigations have reported 21 BBS genes, the product of which are mostly localized to centrosome, basal body or the ciliary transition zone. A combinatorial approach of homozygosity mapping and whole exome sequencing was employed to investigate the disease causing gene. While, segregation analysis was performed through Sanger sequencing. Herein the present genetic report, we analysed two apparently un-related consanguineous BBS families from rural area of district D.I.Khan, Pakistan. Whole exom sequencing in both families determined a recently reported single base deletion mutation c.299delC in 4th exon of *BBS9* gene. The identified frame-shift mutation predictably causes premature truncation of protein product (p.Ser100Leufs*24). This mutation has previously been mapped in a consanguineous Pakistani family, and it is the second report of screening same mutation in two additional families. Nonetheless, the currently recruited families showed wide-spectrum of intra and inter-familial clinical differences. We speculate the evolutionary significance of mutation c.299delC and assume its strong founder effect in Khaisoori tribe of D.I.Khan. Based on these findings, we suggest to develop a molecular diagnostic test that may be used for pre-marital and prenatal screening as a genetic counselling approach.

CYTOGENETIC ANALYSIS OF DOWN SYNDROME THROUGH KARYOTYPING AND Q-PCR

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Human autosomal abnormalities are leading cause of mental retardation. Aneuploidy of autosomal pair 21, widely known as Down syndrome (DS), is among well-known causes of MR. Chromosomal analysis in peripheral lymphocytes of 100 children with varying degree of intellectual disability and those suspected for Down syndrome was carried out to evaluate the cytogenetic profile. Only 13% cases were due to trisomy 21 and could be attributed to autosomal aneuploidy. The frequency of free trisomy; 61.54% when found alone and 76.9% when found along with other chromosome rearrangement is lower and that of Robertsonian translocation (15.4%) and mosaicism (7.69%) was found to be higher when compared to the global data. The data also indicate that certain factors other than maternal age might be playing important role in non-disjunction of chromosome 21 as the mean age of mothers bearing Down syndrome children was only 30 years. Currently, in Pakistan, chromosomal abnormalities are determined by karyotyping which is a time consuming and expensive technique. In the present study, Real Time PCR has been introduced for rapid prenatal diagnosis of aneuploidy like DS. Quantity of DNA in case of Down syndrome was found to be increased.

EPIDEMIOLOGY AND RISK FACTORS OF COLORECTAL CANCER IN PATIENTS VISITING INSTITUTE OF RADIOTHERAPY AND NUCLEAR MEDICINE (IRNUM), PESHAWAR

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Colorectal cancer (CRC) is the most common cancer worldwide. Many factors contribute to the incidence of CRC. Knowing the epidemiology and risk factors of CRC may reduce the risk of CRC. To assess the epidemiology and risk factors of colorectal cancer in patients visiting IRNUM hospital Peshawar a descriptive study was performed. A total of 62 diagnosed cases of cancer of different age groups were included in the study. All patients were interviewed regarding colorectal cancer and information was recorded in a questionnaire. CRC is being more common in mid age group (36-45years, 30.5%). Further the ratios of CRC is higher in females (65.8%) than males (33.6%). The highest percentage of CRC was found in patients belonging to district Charsadda (33.8%). Percentage of colon cancer was 51.6%. Percentage of patients having CRC at stage III was 56.4%. Abdominal discomfort is the most prevalent early symptom of CRC and was found in 88.7% patients. Out of 62 patients 7 (0.1%) patients have a family history of colorectal cancer. Most important risk factor of CRC was found to be the improper diet (40.3%). It is concluded from the current finding that major causes of increase in CRC are the urbanization, modernization in the lifestyle, access to health care and longevity. Furthermore, the risk of colorectal cancer is increased with improper diet. It is more prevalent in females, patients at mid age group and in urban region.

IDENTIFICATION OF METAL RESISTANT CILIATES FROM WATER SAMPLES OF DIFFERENT PONDS

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Urbanization, industrialization and globalization are consequences of rapid increase in population growth. As a result huge amounts of pollutants and contaminants are being produced and discarded into the environment. The contamination of freshwater systems with a wide range of pollutants and toxic heavy metals has become a matter of concern over the last few decades. Bioremediation comprises the use of microorganisms, viable or non-viable, natural or genetically engineered to treat environmental pollution and is typically a cheapest method. The focus of the present study was to identify ciliates (*Paramecium* spp.) from water samples of different ponds in areas of Okara and Lahore. For this purpose four samples of *Paramecium* species were isolated and pure culture was maintained in the lab. AY-1 which was purified from Okara samples was used for further analysis. To optimize the conditions for growth of this *Paramecium* sp., culture was grown at different temperatures (20°C, 25°C, 30°C and 35°C) and pH (6, 7, 7.5 and 8). It showed best growth at temperature 25°C and pH 7.0. Minimum Inhibitory Concentration for Cu²⁺ and Cd²⁺ was checked by growing the isolated *Paramecium* sp. in different concentrations of these metals. In case of copper, maximum resistance was observed at 10mM with 8200 cells/ml at 15th day, while least growth was observed at 30mM with 4900 cells/ml on day 17. Under cadmium stress, maximum growth was observed at 50mM with 7233 cells/ml at 15th day and growth rate showed a decline at 10mM with 5400 cells/ml at 13th day. *Paramecium* sp. exhibited highest no. of cells in the presence of metal as compared to control. Maximum metal uptake for copper was 80% (3.18µg/ml) and for cadmium was 85% (7.40µg/ml) at concentration of 30mM of copper and 50mM of cadmium when incubated for 72 and 24 hours respectively.

ERIOCALYXIN B INDUCES APOPTOSIS IN HUMAN TRIPLE NEGATIVE BREAST CANCER CELLS THROUGH INHIBITION OF STAT3 AND MITOCHONDRIAL DYSFUNCTION

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Eriocalyxin B (EriB), isolated from the *Isodon eriocalyx* plant, is an ent-kaurane diterpenoid with potential anti-cancer properties. Triple negative breast cancer (TNBC) is a highly aggressive form of breast cancer resistant to many common treatments. Signal transducer and activator of transcription 3 (STAT3), which is an oncogenic transcription factor, constitutively expresses in many human malignant tumor, including breast cancer. This study is the first to investigate whether EriB inhibits cell growth and induces apoptosis through suppression of STAT3 activity in TNBC cells. Our results demonstrated that EriB induces apoptosis that was associated with inhibition of NF- κ Bp65 and STAT3, mitochondrial transmembrane potential dissipation, and increased Bax/Bcl-2 ratio and caspase-3 activation. Taken together, our data demonstrate that EriB induces apoptosis via a novel mechanism involving inhibition of NF- κ Bp65 and STAT3 phosphorylation. These results provide the rationale for further *in vivo* investigation and may be a viable compound for developing novel TNBC therapeutics.

ISOLATION AND CHARACTERIZATION OF SPERMATOGONIAL STEM CELLS OF *CYPRINUS CARPIO*

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By realizing, species specific constrains in standardization of procedure for the isolation of spermatogonial stem cells (SSCs), the present study was conducted with the aim to standardize the techniques for isolation and characterization SSCs from juvenile *Cyprinus carpio*. Testis of fish was dissected out and mid, distils and proximal ends were selected for investigation of SSCs. The cut sections were washed with HBSS, minced and incubated with trypsin for three different time period 30, 50 and 60 min. Sample were then filtered through 45 μ m Nylon mesh and cells were studied under 800X and 100X. The SSC type A undifferentiated (Undiff) cells were found largest of all cells with less denser heterochromatin and two to three nucleoli while SSC type A differentiated (diff) cells appeared bit smaller than type A Undiff with oval shape nuclear parenthesis. However, SSC type B (early) were found even smaller with dense heterochromatin and small cytoplasm while SSC type B cells (late) were the smallest of stem cells and almost with negligible cytoplasm. All sub types SSC were found equally distributed all across testis. After gradient centrifugation with percoll, the top layer contained maximum number (40%) of Type A cells followed type B cells (25%). The middle layer contained almost 60% sperm cells and 30% type B cells with very few Type A spermatogonia, while bottom layer consisted of debris of blood cells, sperm cells and dead cells. Furthermore, 50 min incubation appeared best for isolation of highest number of live SSCs.

PATTERN OF BREAST CANCER EXPERIENCE AT LADY READING HOSPITAL PESHAWAR

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Breast Cancer is the commonest malignancy of females all over the world and second leading cause of death due to cancer among females. The aim of this Descriptive study was to see the various features of breast cancer in order to know the pattern of disease in the recent time. The study was conducted from Jan. 2007 to Dec. 2007 in Surgical C Unit, Postgraduate Medical Institute, Lady Reading Hospital, Peshawar, Pakistan. Study included all patients presenting to and admitted in Surgical C Unit LRH, with carcinoma of breast during the above mentioned period. Name, age, sex, other relevant data, history and examination findings and results of histopathology and other investigations were recorded. Total of 46 patients was included in the study, out of which there were 46 female and 1 male patients. Most common age group was 40–49 years with 14 patients, followed by 50–59 years with 12 patients. Most common type of carcinoma was infiltrating ductal carcinoma with no specific features with 38 patients. Other types included 2 infiltrating ductal carcinomas of papillary type, 1 mucinous type and 1 medullary type; 3 invasive lobular carcinomas, and 1 mixed lobular and ductal carcinoma. The disease was left sided in 24 cases, right sided in 20 cases while it was bilateral in 2 cases. Upper outer quadrant of the breast was most commonly involved (n=26). There were 2 cases of stage I, 16 stage II, 20 stage III and 08 cases of stage IV disease. There were 2 cases of grade I, 16 grade II, and 28 cases of grade III. Carcinoma breast is still a common problem presenting at a young to middle age group with invasive ductal carcinoma being the commonest variant with a high grade and a late stage presentation due to lack of screening and awareness programs.

VITAMIN D3-ASSISTED CHEMO-PHOTODYNAMIC THERAPY OF RHODOMAYOSARCOMA CANCER CELLS FOR EFFECTIVE CANCER TREATMENT

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Combination of therapeutic drugs interestingly enhances the treatment outcomes compared to the single agent modality. Vitamin D3 (Vit. D) in the presence of photodynamic drug affects the therapeutic result. Present study demonstrates the Vit-D assisted Chemo-PDT on Rhabdomyosarcoma (RD) cell culture. RD cell line was cultured for the evaluation of each single agent treatment i.e., PDT, Chemo and their combination in the presence of Vit. D. Diode laser ($\lambda = 630\text{nm} \pm 1\text{nm}$, $P_{\text{max}} = 1.5\text{ mW}$) was used as an illumination source. Uptake time of photosensitizer was optimized by means of spectrophotometric measurements. Administered drugs individual and combinatorial response was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. RD culture pretreated with Vit.D3 for 24 hours potentiate sensitivity (10 % ~ 30 %) or set bed for Photodynamic (AlPcS4) and Chemo (Cisplatin (CDDP), Doxorubicin (DOX, D2) or Methotrexate (MTX, D3)) mediated therapy which further adds on by exhibiting synergistic effects ($\text{CI} < 1$). RD culture pretreated with Vit.-D for 24 hours showed encouraging results (%viability) when exposed to CDDP (~50%), DOX (~50%) and MTX (~58%). Post Vit-D+Chemo (CDDP, DOX, MTX) treated culture was followed by PDT and observed more encouraging (~30%, ~20% and ~18%) therapeutic outcomes compared to prior treatment evaluation. Chemotherapeutic agents and Vit-D show significant killing effect and when combine with PDT based on prior measured optimal parameters showed good anti-cancer effects. These results suggest that the employed procedure and sequence may further improve chemo-PDT protocol for cancer treatment.

ENHANCING THE METAL BINDING CAPACITY OF METALOTHIONEIN (MT) ISOLATED FROM *PARAMECIUM SP.*

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Metallothioneins (MTs) are metal binding proteins that are rich in cysteine residues constituting 10–30 % of the total protein, and in which the thiol groups bind to the metal ions. The increasing amount of metal ions in the medium has shown increased production of MTs by different organisms such as bacteria, protozoa and mammals like humans. *PMCdI* is the first gene ever discovered in *Paramecium*, a ciliated protozoan that could produce this MT in response to cadmium. The *PMCdI* syn gene has been cloned in pET41a expression vector and expressed in an *Escherichia coli* BL21-codonplus strain for the first time. Since the gene *PMCdI* amplified from *Paramecium* contained 10 codons, which could act as stop codons during expression in *E. coli*, this gene of 612 bps was synthesized to substitute these (stop) codons for the *Paramecium sp.* specific amino acids. For stability of the expressed protein, glutathione-S-transferase gene was fused with *PMCdI* syn gene and expressed. The cells expressing *PMCdI* syn demonstrated increased accumulation of cadmium. Current study is about expression, purification and characterization of *PMCDI* syn gene and its mutants at atomic and molecular level. So that their metal binding patterns may be studied.

EVALUATION OF COPPER RESPONSIVE *CUSRS* PROMOTER ACTIVATION IN TERM OF *LACZ* GENE EXPRESSION IN RESPONSE TO GOLD

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Copper and gold is known to be essential and non-essential metal respectively for both prokaryotes and eukaryotes. Gold has toxic behavior while copper in high concentration only is known to be toxic for living organisms. One of the operons known for maintaining copper homeostasis is *cus* regulon. It is regulated by two components i.e., *cusS* (sensory) and *cusR* (regulatory). It is bidirectional and regulates both structural and regulatory operons. Promoter *cusRS* from *Klebsiella pneumoniae* was transcriptionally fused in pSL-LacZ vector that was used to transform DH5α. Activation of promoter *cusRS* against gold and copper was studied through the expression analysis of β-Galactosidase; a reporter gene in pSL-LacZ vector. β-Galactosidase expression was confirmed by SDS-PAGE as well as enzyme assay in terms of Miller Units using ONPG as substrate. Promoter *cusRS* was found to be activated in the presence of both copper and gold. Though, it was more activated against copper than gold. Additionally, expression analysis was also correlated with intracellular copper and gold concentrations. Initially, increased concentration of each metal resulted in more activation of promoter *cusRS*, however, concentration near MIC resulted in deactivation of promoter *cusRS*. Sequence of promoter *cusRS* was analyzed on homology basis for the presence of CusR binding box among different species belonging to family Enterobacteriaceae.

ASSOCIATION OF CONSANGUINITY AND ABO BLOOD GROUPS WITH B-THALASSEMIA MAJOR IN DISTRICT DADU AND HYDERABAD, PAKISTAN

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Consanguinous marriages are the deeply rooted social trend in Pakistan, which lead to the high prevalence of inherited diseases. Thalassaemia is an inherited disorder characterized by the abnormal synthesis of hemoglobin leading to anemia. β-thalassaemia is the most common inherited disorder in Pakistan particularly in Sindh. The

patients of β -thalassemia need repeated transfusion, which might cause complications causing the high rate of mortality in blood transfusion dependent β -thalassemia patients. Several studies have previously reported the high prevalence of β -thalassemia in Pakistan, however, the prevalence of β -thalassemia in the siblings borne from β -thalassemia carrier patients and association of ABO blood group with β -thalassemia have not been extensively studied. The purpose of this study was to find out the prevalence of β -thalassemia in the siblings of β -thalassemia carriers parents. This study was also set to find out the association of β -thalassemia with ABO blood groups. This was a cross sectional study carried out from August 2016 to July 2017 in the Thalassemia centers of district Dadu and district Hyderabad. In this study, 50 families were selected for the study. The results indicate that almost half of the births 44.64% from β -thalassemia carriers are the β -thalassemia patients. B blood group was found to be higher in both in male and female β -thalassemia patients. Male had higher provenance of β -thalassemia than female patents. The pattern of ABO blood group distribution was $B > O > A > AB$, and for the RH factor it was $RH +ve > Rh -ve$. Conclusively, this study indicates the higher prevalence of β -thalassemia in siblings borne from β -thalassemia carrier's parents. β -thalassemia was higher in male patients and B blood group was higher than other blood groups.

3. HUMAN AND ANIMAL DISEASES

CAUSES OF AMENORRHEA IN HYDERABAD SINDH PAKISTAN

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Prevalence of the problem of amenorrhea is going to become very common especially in the urban areas. In this project it was to investigate the actual cause of the prevalence of the problem especially in Hyderabad city. In this context, 25 patient suffering from this problem were interviewed at civil hospital Hyderabad Liaquat university of medical health science Information sought was related to the type of Amenorrhea, Living status, medication /contraceptives being used by the patient, feeding habits and the hormonal problem if any or the obesity, As per data collected, maximum patient 16 out of 25 had secondary Amenorrhea. The living status of 15 patients out of 25 was apartment whereas, rest of patients were living in the open house /congested houses. As far as the medication is concerned it has noted that majority of the patients 18 out of 25 used contraceptives. Most of the individuals 19 out of 25 were taking home made food and remaining 6 patients were frequently using junk food. 19 out of 25 were obese whereas, rest of the patients were suffering from PCOS /Thyroid problem .It is concluded from the results mentioned above that, Most of the amenorrhea patients having secondary Amenorrhea . The patient especially living in the apartment may suffer from such a problem. The uses of contraceptives also play a main role in the prevalence of the Amenorrhea. Obesity is also the main reason of the occurrence of the Amenorrhea.

PREVALENCE OF GASTROINTESTINAL DISEASES IN HUMAN CAUSED BY HOUSEFLY (*MUSCA DOMESTICA*) VECTOR IN DISTRICT HYDERABAD

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Housefly (*Musca domestica*) is closely associated with people in all over the world. It feeds on human food items and transmits the number of diseases to human, they carry disease causing agents from human and animal wastes and transport to human. Housefly carry pathogens on their bushes, legs and small hairs that cover their body, in addition to housefly other fly species live in human settlement and cause harmful impact. The present survey was conducted in different hospitals such as LUMS hospital, Hillel-e-Ahmer, Shah bhittai government hospital, and Wali bhai rajputana hospital of district Hyderabad from January- August. We have found 69 patients of cholera, 1730 patients of diarrhea, 780 patient of food poisoning and 183 patients of dysentery. In addition to collection of patients data we also surveyed the various localities of Hyderabad for the observation of housefly occurrence in those localities, 734 specimens of houseflies were collected and brought to entomological laboratory for identification of housefly species. Our results showed that only one species of housefly, *Musca Domestica* was present in studied locality therefore this species was playing role in transmitting gastrointestinal diseases in studied area.

ESTIMATION OF MALONDIALDEHYDE AND CIRCULATING BIOCHEMICAL MARKERS IN HEPATITIS C (HCV) PATIENTS RECEIVING INTERFERON

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The most common cause of liver diseases is Hepatitis C virus. Cirrhosis, chronic liver diseases, liver transplantation and hepatocellular carcinoma diseases are lead by hepatitis C virus (HCV). Glutathion-peroxidase (GPx),

catalase (CAT) and superoxide-dismutase (SOD) are the important antioxidant cellular enzymes. Each enzyme is located in different compartments of the cell and is involved in different ROS reduction processes. To estimate the MDA and Biochemical response in HCV patients receiving interferon. Comparative Study. 5.0 ml blood sample of 60 diagnosed HCV patients taking interferon and 50 age and sex matched healthy individuals was taken from vein in clotted gel vials from hepatology and gastroenterology department of Mayo hospital and Jinnah Hospital Lahore. The estimation of MDA, CAT, GSH, SOD, NO and Micronutrients were estimated. The spectrophotometric reading of samples portrays that MDA level in HCV patients is remarkably elevated (5.21 ± 0.33) than normal person (1.29 ± 0.21). The value of GSH in interferon taking HCV patients reduced (0.19 ± 0.12) as compared to normal individual (6.32 ± 0.13). The CAT level was decreased in patients (2.80 ± 0.03) while the value of healthy individual is high (4.11 ± 1.02). Results parade the amount of SOD in HCV patients (0.50 ± 0.19) it was high in normal people (2.12 ± 0.22). Patients of HCV has remarkably deficient vitamin A (1.38 ± 0.13) (1.38 ± 0.13) than normal person (7.14 ± 0.43). The outcome obtained revealed the worth of significant ($P=0.000 < 0.05$). Reduced level of Vitamin E in diseased person (2.30 ± 0.34) than normal (4.33 ± 0.95). So it depicts that HCV patients shows the highly significant behavior ($P=0.000 < 0.05$). The present study expresses the enlarged oxidative stress during hepatitis C (HCV) and the possible mechanisms of its proliferation. Hepatitis C (HCV) infection is characterized by the increased oxidative stress.

OCCURRENCE OF DIARRHEA DISEASE TRANSMITTED BY HOUSEFLY (VECTOR/ MECHANICAL CARRIER) IN HYDERABAD DISTRICT, SINDH PAKISTAN

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Housefly (*Musca domestica*) is the notorious mechanical vector associated with animals and human, which create harmful impacts. Housefly carry pathogens on their bushes legs and small hairs that cover their body and act as carriers of various diseases including Diarrhea. Diarrhea is very common vector borne disease in the area of Hyderabad district due to unhygiene condition, poor sanitation and unawareness among the people about this disease and the ratio of vector borne has been increased. So the present study was carried out from July to September on various localities of Hyderabad in order to monitor the occurrence of Diarrhea in this region. We surveyed main four hospitals of different localities of Hyderabad to collect the data of Diarrhea disease. Overall we collected the data of 1453 diarrhea patients, out of these, 453 were from LUMHS hospital, 375 from Hilal-e-Ahmer, 453 from Shah Bhattai government hospital and 172 patients were reported from Wali Bhai Rajputana hospital. We found that the ratio of male patients was higher as comparison to the female patients. After collection of patients' data we surveyed these localities for collection of housefly to observe the ecological behavior of housefly.

COMPARATIVE OCCURRENCE OF HEPATITIS B IN LOCAL POPULATION OF DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Pakistan carries one of the world's highest burdens of chronic hepatitis and mortality due to liver failure and hepatocellular carcinoma. In Pakistan about 10-12 million of the people are suffering from hepatitis. It is estimated that there are about 400 million of carriers of HBV all over the world. This study is conducted in the Bacha Khan Medical Complex Swabi. A total of 3,977 patients, belonging to different localities were screened for HBsAg by using ELISA (enzyme linked immunosorbent assay) and ICT (immuno chromatographic technique). Out of 3,977 cases, 81 (2.03%) were positive for HBV. The common symptom noted among most of the patients was loss of appetite (65%). Co-infection was found only in 6 patients. The common risk factors associated with HBV was found such that history of dental treatment was 70%, treatment from unqualified doctor 49%, history of blood transfusion 43%, history of minor/major surgery 40%, HBV positive patients in family 35%, shaving from street barbers 32%,

diabetes 16%, tattooing 11%, HCV co- infection 7.41%, sharing of drug injecting equipments 1.2%, HIV and T.B co-infection was 0%. The highest %age was noted in the area of Swabi (0.58%). The +ve patients was mostly young adults of age range from 21 to 40 years.

COMPARATIVE ANALYSIS OF PHYSIOCHEMICAL ALTERATIONS IN DIABETIC AND NON-DIABETIC SUBJECTS IN DISTRICT SIALKOT, PAKISTAN

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Important biochemical factors and physical risk factors were considered and analyzed by comparing 100 Diabetes mellitus (DM) patients and 100 control subjects. The significant biochemical factors for instance cholesterol, triglyceride, low density lipoprotein, high density lipoprotein, fasting blood glucose as well as the physical risk factors such as body mass index, systolic blood pressure, diastolic blood pressure, fat consumption, sugar consumption, physical activity, staple food were analyzed. The mean of total levels of biochemical parameters of patients were cholesterol level (213.19 ± 4.96 mg/dL), triglyceride (239.80 ± 6.35 mg/dL), low density lipoprotein (118.88 ± 4.22 mg/dL) high density lipoprotein (43.87 ± 0.87 mg/dL), fasting blood glucose (201.92 ± 6.83 mg/dL) as compared to control group (180.35 ± 5.58 mg/dL, 201.21 ± 8.00 mg/dL, 85.02 ± 4.30 mg/dL, 60.15 ± 3.74 mg/dL, 95.10 ± 1.24 mg/dL respectively. The prevalence of HDL was about low in the DM patients than control and it was most highly significant. The frequency of total cholesterol and HDL was higher in male patients than the female patients, while the level of TG, LDL, and FBG was presenting not any significant difference in male and female patients. The prevalence of HDL was lower in patients than control groups. In patient's male and control male gender showed a significant difference between total cholesterol and total triglyceride that was (0.4764) and (0.0229). The risk factor like familial blood pressure, familial diabetes mellitus, fat consumption and sugar consumption between patients and control subjects show equal effects.

PREVALENCE AND SUSCEPTIBILITY PATTERNS OF BACTERIAL INFECTIONS IN CHILDREN FROM CLINICAL SAMPLES IN ISLAMABAD AND RAWALPINDI

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Bacterial infections execute great burden on medical structures because of great incidence of community-acquired and nosocomial infections in children. Occurrence and prompt blowout of resistance producing enzymes is becoming a most important communal healthiness disaster globally, as well as accountable for huge extent of infections. To combat this evolving resistance a constant investigation of antibiotics vulnerability design of bacterial infections in children is vital at local levels that can then afford support in management of operational preliminary treatments. Clinical isolates of bacteria were studied in terms of sample origin and demographics. The data was statistically analyzed using Chi-square (χ^2) test, confidence interval (CI) and odds ratio analysis for paired samples. The multiple antibiotic resistance index (MARI) of different antibiotics against isolated bacteria was calculated. The overall prevalence of bacterial infections was predominantly observed in female patients. A difference in the antibiotic susceptibility profiles of *Enterobacteriaceae*, *Non-Enterobacteriaceae*, *Staphylococcus spp.*, *Enterococcus spp.*, and *Streptococcus spp.*, was observed. The predominance of bacterial infections has been found in females. TAC, DAL, VAN, MET, GEN and CRO were found the highest resistant drugs. However, AMP, CFM, FOS and CXT were found the most susceptible drugs showed the highest sensitivity.

INSPECTION OF BETA-THALASSEMIA MAJOR PATIENTS, UNDERGOING TERTIARY CARE AT A LOCAL THALASSEMIA CENTRE IN PAKISTAN

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Beta thalassemia is one of the most common autosomal recessive disorders of haemoglobin. Despite prevalence around the world, it is most common in the Mediterranean Basin, Southeast Asia and Africa, and if left untreated it can lead to different secondary complications such as Ascites, Jaundice, splenomegaly, hepatomegaly and hepatitis. The current prospective study was carried on 177 patients; their files were analysed to check the patient history, age, gender and physical examination like pallor, hepatomegaly, ascites and splenomegaly. Antisera was used to detect ABO and Rh blood group, haemoglobin electrophoresis for haemoglobin variants, sysmex analyser for Complete Blood Count (CBC), AxSYM instrument for ferritin level and ELISA for HCV. Among 177 patients, 111 (62.71%) were males and 66 (37.29%) were females with an average age of 8.62 years. The mean age of diagnosis of β -thalassemia was 1.2 years after birth and the average age of first transfusion was 1.3 years. Blood group "O" was the most prevalent blood group in the studied population. Average Hb, HbA, HbA2 and HbF were 6.57, 29.16, 5.91 and 62.42 respectively. Ferritin level among all 177 patients was more than the normal range, with a mean Ferritin level of 2806.22 μ g/L. examinations for secondary complications revealed that Pallor was the most common disease, having a prevalence of 79.66%, followed by Splenomegaly (64.9%), Hepatitis C virus (17.5%) and hepatomegaly (9%). The current study will be fruitful for the management and control of thalassemia patients, undergoing tertiary care at local hospitals of Pakistan.

ANTIBIOTIC RESISTANCE PATTERNS OF EXTENDED SPECTRUM β -LACTAMASES PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION IN HOSPITALS OF ISLAMABAD

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Urinary tract infection (UTI) is one of the most common diseases in adults. Early diagnosis and comprehensive treatment can significantly decrease late serious complications. Beta-lactam antibiotics are the most commonly used treatment of bacterial UTIs. However, the development of multi-drug resistant extended-spectrum beta-lactamase producing bacterial strains has greatly complicated the treatment of UTIs. This study was conducted to determine the prevalence and antibiotic resistance pattern of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among UTI patients belonging to different sexes and age groups in Islamabad. A total of 340 bacterial isolates were collected from UTI patients visited or admitted at Pakistan Institute of Medical Sciences (PIMS), Government Services Hospital (Polyclinic) and Capital Development Authority (CDA) Hospital Islamabad, with UTI during January, 2018 to September, 2018. These isolates were screened as ESBL-producers against any one or more of the ceftazidime, cefuroxime, cefotaxime and cefpodoxime by double disc diffusion. Afterwards, the ESBL phenotype was confirmed using cephalosporin/clavulanate combination discs. Antibiotic resistance pattern of these isolates was determined by employing Kirby-Bauer disc diffusion method and the inhibitory activities of resistant isolates were tested by Minimum Inhibitory Concentration (MIC) test, using the NCCLS protocol. In the present study, a high proportion of *E. coli* was identified among the ESBL-producing isolates as compared to *K. pneumoniae*. Majority of the patients were females (56.69 %). A higher percentage (53.57 %) of patients aged ≥ 65 years, in both sexes. In general, higher rates of antibiotic resistance were found in ESBL-producers as compared to non-ESBLs. Both ESBL-producing *E. coli* and *K. pneumoniae* showed higher resistance rates to penicillins, sulfa-drugs, cephalosporins, quinolones and chloramphenicol (60-100%). Meropenem was found to be the most effective drug against both ESBL-producing and non-producing strains of *E. coli* and *K. pneumoniae*. Nitrofurantoin and amikacin were also relatively effective against *E. coli* and *K. pneumoniae*. Higher MIC values indicated an increasing trend of resistance in these uropathogens. The rates of antibiotic resistance were found higher in ESBL-producers as compared to non-

ESBLs. A high resistance rate against most of the tested antibiotics has revealed a high antibiotic pressure that may be attributed to antibiotics misuse and self-medication, therefore, prudent use of these antimicrobial agents is advised to prevent or minimize the development of resistant strains.

MOLECULAR DIAGNOSIS OF X-LINKED CHRONIC GRANULOMATOUS DISEASE IN PAKISTANI PATIENTS

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Chronic granulomatous disease (CGD) is a primary immunodeficiency (PID) caused by mutations in the five structural genes (CYBB, CYBA, NCF1, NCF2, and NCF4) of phagocyte Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that typically results in a decrease in function or inability to generate a respiratory burst, leading to defective killing of pathogens, including fungi and intracellular bacteria. Mutations in CYBB, encoding the gp91phox (also known as NOX2) result in X-linked CGD account for approximately 65% of CGD cases. Here, for first time we aimed the molecular identification and clinical characterization of genetic mutations in Pakistani patients with X-linked CGD. Relevant clinical data of 50 Pakistani male patients who were highly suggestive of having X-linked CGD by clinical history was reviewed. Oxidative burst and NADPH protein expression was evaluated by flow cytometry, while Genetic analysis was performed by Sanger sequencing. The pathogenicity prediction of novel mutations was performed by bioinformatics predictor tools. We thus report the absence of an oxidative burst in the phagocytes of the 35 patients. Among these patients, the genetic diagnoses of 19 patients revealed heterogeneous mutations in the CYBB. Pakistani X-linked CGD patients presented a wide range of clinical manifestations most frequently bacterial and fungal infections. Our work expands the genetic spectrum of X-linked CGD and creates awareness among physicians about CGD. The aims of this research on CGD are to save lives by definite diagnosis which guides physicians to initiate the specific treatment.

PREVELANCE OF HEPATITIS C VIRUS IN DHQ HOSPITAL SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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The beginning of hepatitis C virus (HCV) can be conceptualized at numerous stages. HCV very common in Swabi due to poor hygienic environmental condition and due to low socio-economic values initially, backgrounds strength mention to its theatrical binge during the twentieth century. As plasma born. Medicinal actions, immunization, blood transfusion and extra newly vaccinating drug use. Hepatitis is deadly viral infection caused by hepatitis c virus it is the major cause of chronic liver diseases. Cirrhosis, hepatic cellular and development of hepatocellular carcinoma. it accounted for 23% of viral acute disease and of chronic liver diseases the present study were conducted in Swabi DHQ hospital for last two years 2015 to 2016 during (2017). The data collected in the of \months of Jan and Feb(2017). Out of 4000 patients 1500 were infected by HCV and 2500 were non HCV. In the month of Jan (2017) 880 patients were screened out of 1500 while 1000 were non HCV cases. In month of Feb (2017) 620 patients were screened out of 1500 while 1500 were non HCV cases. Patients belonging to various areas of Swabi were tested for anti-HCV by using immunochromatographic technique and confirm conducted in the laboratory of district head quarter hospital in Swabi.

IN VITRO ANTICOCCIDIAL POTENTIAL OF *TRACHYSPERMUM AMMI* EXTRACT ON OOCYSTS OF DIFFERENT *EIMERIA* SPECIES OF CHICKEN

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In current experiment *in vitro* anticoccidial potential of *Trachyspermum ammi* (Ajwain) extract was evaluated. For this purpose, an *in vitro* sporulation inhibition assay was used. Collected oocysts of different *Eimeria* species of chicken were exposed to six different concentrations (w/v) of *Trachyspermum ammi* extract (TAE) in 10% Dimethylsulphoxide solution (DMSO). Dimethylsulphoxide (DMSO) and Potassium dichromate solution (K₂Cr₂O₇) served as control groups. Results of study revealed that *Trachyspermum ammi* extract (TAE) showed inhibitory effect on sporulation (%) and damage (%) of *Eimeria* oocysts in dose dependent manner as compared to both control groups. *Trachyspermum ammi* also damaged the morphology of oocysts in terms of shape, size and number of sporocysts.

SENSITIVITY OF *SALMONELLA* OF CHICKEN AGAINST GARLIC

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Salmonella is quite prevalent and one of the major threats to poultry industry in Pakistan. Mortality may vary from negligible to 10-20% to 80% or high in severe outbreaks. To avoid antibiotic residual toxicity, this study was made. A total of 40 sensitivity tests were made for the salmonella isolated from the 83 diseased birds showing the necropsy lesion of *Salmonella* infection. Collected sample from liver, lungs ceca and kidney, were cultured on Nutrition Agar and Brilliant Green Agar (BGA). The serotypes were recognized as *Salmonella Pullorum*, *S. Gallinarum*, *S. Pamona* and *S. II*. Sensitivity dishes were prepared from garlic juice in 100%, 75% and 50% concentration and their efficacy was tested in the same quantity (0.03ml). Efficacy of garlic was tested in different quantities i.e. 0.01ml, 0.02ml and 0.03ml in 100% concentration. In 37 (92.5%) of these tests, *Salmonella* was sensitive to the tested quantity of garlic.

THE COMPARATIVE STUDY OF PREVALENCE OF TOXOPLASMA INFECTION IN PREGNANT FEMALES OF DISTRICT PESHAWAR AND MARDAN, PAKISTAN

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Toxoplasma gondii is a species of parasitic protozoa in the genus *toxoplasma*. Cat is the definitive host of *T. gondii* but this parasite can be transferred to the warm blooded animals such as humans. It acts as a human pathogen. Infected pregnant women transmit the disease to their offspring and cause serious problems in fetuses. The aim of this work is to study the comparative prevalence of toxoplasmosis in pregnant females in district Peshawar and Mardan. The study was carried out from June to August 2018. Total of 140 patients were screened in which 70 patients from LRH (Peshawar) and 70 from DHQ hospital (Mardan). Toxo IgG/IgM rapid test device were used in both hospital to detect the *T. gondii* infection. In Peshawar out of 70 patients, 17 patients were positive *T. gondii* infection while 53 were negative. While in Mardan out of 70 patients, 29 patients were positive for toxoplasmosis disease and 41 were negative for disease. Women with the age groups of 26-35 are at high risk. It is concluded that

toxoplasmosis disease is higher in Mardan as compared to Peshawar. The main risk factors are found to be lack of awareness about disease, lack of hygienic condition and use of undercooked meat. It is recommended that there is a need of awareness about toxoplasmosis disease in general population. Pregnant females prevent themselves from those materials which are contaminated such as soil, water, raw vegetables, unpasteurized goat milk, undercooked meat and cat's feces.

SERO-PREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS AND ASSOCIATED RISK FACTORS IN DAIRY HERDS OF KHYBER PAKHTUNKHWA PAKISTAN

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Bovine Viral Diarrhea (BVD) is measured one of the most frequent and economically most important viral infections in cattle in various countries around the globe. Epidemiological investigations have revealed a prevalence of 0.5 - 02 percent of BVDV persistence in the bovine population in different countries around the world. It is associated with reproduction, respiratory and gastrointestinal diseases of cattle. In addition, this virus can also cause infections in sheep, goats, camels and pigs, etc. Financial losses related with BVDV infection seems to be primarily due to problems related to reproduction. BVDV mostly leads to failures of reproduction, abortion, mummification, still birth, persistency and other secondary infectious problems by other pathogens. Bovine Viral Diarrhea (BVD) is one of the most frequent and economically important viral infections in cattle in various countries around the world. It is associated with reproductive, respiratory and gastrointestinal diseases of cattle. Planned sampling regarding risk factors and prevalence of bovine viral diarrhea virus (BVDV) in Pakistan especially in Khyber Pakhtunkhwa is scarce. This study was conducted to determine sero-prevalence of BVDV and associated risk factors in dairy herds of Khyber Pakhtunkhwa province. Districts Peshawar, Mardan, Kohat, Bannu and Dera Ismail Khan of Khyber Pakhtunkhwa were selected for sampling. A total of 300 samples from cattle (n=150) and buffaloes (n=150) were collected from 30 different dairy herds. All the samples were subjected to ELISA to determine presence of antibodies against BVD virus. Out of all processed samples, overall 12.66% were positive against BVDV. Out of 150 samples of cattle, 27 samples (18 percent) showed sero-prevalence against BVD virus and, 7.33 percent (11/150) positive cases were observed in buffaloes. Questionnaire was designed and filled to evaluate risk factors associated with BVDV prevalence. Data was statistically analyzed by using chi square test. It was observed that isolation of newly purchased animals (P=.014), workers visiting other farms (P=.013), mixed farming (P=.015) and veterinary services (P=.009) were potential risk factors associated with BVDV. Study concluded that enactment of control and deterrence actions among farmers, with objective of averting propagation of agent in the herds, is necessary. Distinctive consideration should be given for addressing identified risk factors of BVDV, such as checking status of BVDV before allowing entry of newly purchased animals into herds, discouraging the workers visiting other farms and encouraging culling of animals with more abortion history.

EVALUATION OF BIOCHEMICAL AND ANTI-OXIDANT STATUS IN BETA-THALASSEMIA PATIENTS FROM LAHORE, PAKISTAN

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β -thalassemia, one of the two primary kinds of thalassemia, is a typical hereditary issue. Transformations, influencing the different levels of β -globin quality articulation causing β -thalassemia, have been found in an overwhelming number. The most debilitating life-restricting complexity caused by press over-burden in beta-thalassemia patients is heart malady caused by myocardial siderosis and 71% of beta-thalassemia patients kick the bucket with these cardiovascular difficulties. The objective of present study was to evaluate the biochemical and anti-

oxidative status in patients suffering from thalassemia (RA). The spectrophotometric reading of samples revealed that MDA level in thalassemia patients was remarkably elevated (3.02 ± 0.45) while it was 1.29 ± 0.21 in healthy individuals. The level of GSH was decreased (0.18 ± 0.14) as compared to normal individual (6.32 ± 0.13) whereas the CAT level was moderately elevated in patients (2.64 ± 0.11) than normal person (4.11 ± 1.02). The results also showed that SOD was elevated in patients (5.33 ± 0.81) than normal people (3.21 ± 1.07) while the Vitamin E level in the patients was lower (2.38 ± 0.59) than normal person (4.33 ± 0.95). Red Blood Cells also decreased in thalassemia patients (18.61 ± 3.32) as compared to normal (39.8 ± 7.19). Serum Iron level was elevated remarkably in patients (131.33 ± 2.56) while in healthy individuals it was 93.21 ± 1.11 . The results indicated that the data was statistically significant at $P=0.000 < 0.05$. The expanding level of MDA demonstrated possible high rate of lipid peroxidation in thalassemic patients. Decreased level of RBC, hemoglobin and overloaded iron were probably associated with the pathogenesis of thalassemia.

INFECTIOUS DISEASE EPIDEMIOLOGY: SCOPE AND SIGNIFICANCE IN CONTEXT OF CREATING FOCUSED RESEARCH ROADMAP

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The dynamics of disease spread within any population depend on a number of discrete variables. Understanding of these variables are collectively measured under Epidemiology, which is imperative for effective targeted disease control. Pakistan faces an extremely grave situation with respect to the status of infectious disease influx. The related morbidity and mortality is creating severe socio-economic burden for the general population. However, there is a serious lack of accurate reliable documentation and surveillance systems in place to monitor disease spread (disease epidemiology). Recently Government of the Punjab has launched an initiative to monitor and record disease spread for some major vector borne infectious diseases and are likely to include other diseases in near future. The aim of this review is to assess the current situation of targeted epidemiological research conducted in case of infectious diseases. Before digging into molecular aspects of disease pathogenesis, investigations into its epidemiological aspects should be prioritized. This will allow the policy makers to put preventive and eradication measures in place with the help of data generated by the researchers. In resource limiting settings of Pakistan, this way researchers can have some direct impact on the betterment of general public health.

PREVALENCE AND ANALYSIS OF HEPATITIS C GENOTYPES IN PUNJAB AND KPK

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Hepatitis is the major cause of liver diseases throughout the world. It is one of the major health issues in third world countries especially Pakistan and Egypt. Types of Hepatitis vary from Hepatitis A to Hepatitis E where Hepatitis A and E are just seasonal. It is necessary to establish regional laboratories and healthcare centers to estimate exact burden of Hepatitis in Pakistan. Hepatitis C virus is an enveloped RNA virus. It has a diameter of 50nm. It is classified from the genus Hepacivirus and form the family named as Flaviviridae family. HCV virus is classified into 6 genotypes (1-6) that are further divided into subtypes as (a, b, c etc). They are classified according to genomic sequence and heterogeneity. The most common and widely found Genotypes are (1-3) and the subtype 'a' and 'b' are most common. Previously, Interferon's were used for Hepatitis C treatment along with some oral drugs but these drugs are not that widely used now. An oral drug has now been used to treat hepatitis C under different brand names most common of which is Sovaldi (sofosbuvir). Cure rates for that are 30 to 95%. Response rate depends upon different factors like type of virus, immune system of individuals, hygienic conditions etc. Relapse rate is also less than other treatments being in used. Current study was done to find out the prevalence of different HCV genotypes in

local population of Punjab and KPK province. Blood samples are received from different parts of Punjab and KPK through courier.

GENES INVOLVED IN OSTEOARTHRITIS

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Osteoarthritis (OA) is a painful, unbearable aching disease characterized by loss of articular cartilage with contemporary changes in other tissues of the synovial joint. OA results from an unevenness between catabolic and anabolic factors, and biologic agents either target specific catabolic proinflammatory mediators, such as cytokines, nitric oxide synthesis, or affect anabolism more generally. Genetic studies have shown that a number of common gene variants increase the risk of developing osteoarthritis like IL-6 that is detected in synovial fluid and expressed in osteoarthritic cartilage which makes its inhibition an appealing potential target in the treatment of OA. MCF2L gene and protein expression are detectable in joint tissues, with quantitative differences in the expression of the gene. 21 MCF2L targets protein coding transcripts were recognized. Strategies in OA therapy need to be reconsidered and new molecules emerging from preclinical data should focus on treating the early phase of the disease where damage may be reversible, and treatment should be modified to fit each patient.

COMPARISON OF GENEXPERT AND SOLID CULTURE MEDIA IN THE DIAGNOSIS OF *MYCOBACTERIUM TUBERCULOSIS*

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Tuberculosis is one of the predominant infectious diseases causing significant deaths worldwide. Detection of *Mycobacterium tuberculosis* using culture media was officially recognized by WHO in 2007. However, there is significant lacking in authentic evaluation for its effectiveness on clinically important attributes. Gene expert detects the presence of *M. tuberculosis* based on molecular profile and also reveals the resistance of Rifampicin drug. It differentiates DNA to detect live bacilli and use Real-time PCR to amplify rpoB quality in *M. tuberculosis*. Culture media gives more reliable results when compared with GeneXpert and is considered gold standard in detection of *M. tuberculosis*. The current study was designed to study the comparison of GeneXpert and solid culture media. Sputum samples of 250 patients were investigated using both techniques. 30 samples out of 250 showed positive results while 220 showed negative results with culture media. Only 17 samples showed positive results with GeneXpert while 233 showed negative results. Culture test reveals positive growth of *M. tuberculosis* while GeneXpert do not often. Culture test and GeneXpert are not equally efficient in detection of *M. tuberculosis*. The findings of current study showed that culture based detection method for *M. tuberculosis* is more efficient, sensitive and reliable than GeneXpert.

VIRAL HEPATITIS IN DISTRICT QUETTA: DEMOGRAPHIC FEATURES AND VACCINATION RECORD

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Hepatitis is an inflammation of the liver, viruses cause most cases of hepatitis. In this study, we have collected the data of total 1580 ELISA positive patients affected with viral hepatitis, who visited the Bolan Medical College (BMC) hospital and a clinic present at Patfeeder, Quetta, during January 2017-July 2018. A Questionnaire was

prepared to take the information of patients and the data was then analyzed. HCV, HBV and HDV patients were more prevalent than any other viral hepatitis. It was observed that HBV is the most prevalent among both genders. Males have more chances of HCV/HBV, HDV and mixed ($p < 0.05$). Married males and females had HCV and HBV and they belonged to rural areas ($p < 0.05$). Patients under treatment were less as compared to new patient entries and the patients who attended the BMC for 1st vaccination were more prevalent than who attended for 2nd or 3rd time during 2017. Only 26% of the patients were found ELISA positive either for HCV, HBV or HDV, it means doctors are sending a large number of patients in diagnostics lab for HCV, HBV and HDV diagnosis. Male Patients of age group 46-60 were more prevalent in viral hepatitis while females had non-significant differences in different age groups. So, HCV and HBV prevalence rate is increasing day by day. It is necessary to spread the awareness among the persons how to prevent from this deadly disease. Also proper vaccination and treatment programs should be there to decrease the death rate. Government should pay more attention on poor persons and allocate a good amount of budget for treatment of poor persons.

ROLE OF AMINOACYL-tRNA SYNTHETASES GENE IN EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE 29 IN PAKISTANI POPULATION

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Epilepsy is one of the most common neurological disorders and is more common in developing countries as compared to developed one. Epilepsy has a various types of phenotype depends upon the pathways involved in the signaling process. Epilepsy is a group of neurological disorders characterized by recurrent epileptic seizures. Genetics is believed to be involved in the majority of cases. During the last decade, many genes and mutations associated with epilepsies have been identified. To find out the genuine cause of the epilepsy in Pakistani idiopathic epilepsy patients was the major factor behind this project. Five patients suffering from early infantile were selected on the basis of the clinical findings. Although it is understood that SCN1A gene is the most causative factor for genetic epilepsies and majority of the cases of early infantile are found to be responsible by SCN1A gene. In current study Central Punjab from Pakistan was selected for the identification of Epilepsy Patients to conduct molecular study for now and future. Epilepsy Questionnaire (from NINDS) was used which is the short form of the Questionnaire. Whole Exome sequencing was the key technique to find out the molecular alterations. Aminoacyl-tRNA synthetases (AARS) are indispensable enzymes in protein production because they allege tRNAs with their cognate amino acids. In this study we found that Epileptic encephalopathy, early infantile, 29 (EIEE29) [MIM:616339] is caused by the mutation in AARS gene.

OCCURRENCE OF HEPATITIS B, C AND HIV AMONG BLOOD DONORS AT DIFFERENT HOSPITALS OF LAHORE

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Blood is one of the major sources of transmission of Hepatitis B, Hepatitis C, HIV and many other diseases. Keeping in mind the significant risk of acquisition of HBV and HCV associated with transfusion of blood or plasma derived products, the Government of Pakistan in 2003 introduced a National Blood Transfusion Policy to enable proper screening of blood prior to recommending it for transfusion. The objectives of the study were to find out the frequency of hepatitis C, hepatitis B and HIV infection among blood donors at different hospitals of Lahore; to understand the relationship of demographic factors and various other risk factors contributing to the positive cases of Hepatitis C, Hepatitis B and HIV; and to disseminate the findings of the study for the constitution of screening program at Blood Transfusion Centers. It was cross-sectional study in which 200 blood donors coming to blood bank

of four hospitals of Lahore i.e. Lahore General Hospital, Services Hospital Lahore, Mayo Hospital Lahore and DHQ Kot Khawaja Saeed Hospital Lahore participated. The data collected through questionnaire was analyzed by using SPSS. Among 200 blood donors, 37.5% were 26-30 years old. Most of them were male (90.5%). 81.5% had previous history of blood donation. 6.5% had history of drug addiction. 44.5% were professional blood donors. 1.0% had history of sexual contact with sex worker infected with HCV, HBV & HIV. Other than these the history of jaundice, shaving from barbers, nail cutter sharing, tooth brush sharing, surgical intervention, needle stick injury, I/V injections/drips and tooth extraction/dental procedure was recorded. A mainstream (71.5%) had no history of surgical intervention. Result shows that 10.5% blood donors were Anti-HBV positive, 12.5% were Anti-HCV positive and 0.5% was HIV positive. To overcome the problem of hepatitis B, C and HIV transmission, health education programs should be held among blood donors and general public to prevent them from infection.

PREVALENCE OF DIABETIC MELLITUS IN HEPATITIS C PATIENTS IN TEHSIL WAZIRABAD, DISTRICT GUJRANWALA PAKISTAN

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Hepatitis is an inflammation of liver. The condition of liver's inflammation can be self-limiting or there are certain chances that it may leads to liver cancer, fibrosis or cirrhosis. The main causes that lead to hepatitis in the world are mostly hepatitis viruses but some toxic substances that cause infections include certain drugs, alcohol and various autoimmune diseases. As diabetes and hepatitis are most common diseases in Pakistan. A cross sectional study was conducted in Tehsil Wazirabad, District Gujranwala to analyse the percentage of patients having hepatitis C had the risk of diabetes mellitus. People suffering from hepatitis C have predominant chances to develop diabetes mellitus as the HCV virus participates in pathogenesis of type II diabetes mellitus. HCV virus causes pathogenesis in two ways either directly destroys the β cells of pancreas or contribute to the specific autoimmunity of β cells. For this research work, a questionnaire was developed and data was collected from hospital. Data were then analysed by using statistically designed software. A total of 29.33% patients, suffering from hepatitis C, were found to be diabetic in Tehsil Wazirabad. 14.70% male and 38.59% female patients suffering from hepatitis C were diabetic. Our results suggest that patients of C are at higher risk to develop diabetes therefore; we supposed that persons having hepatitis C should regularly visit doctors for routine check-up of diabetes and change their life style to reduce the risk of developing diabetes.

4. MICROBIOLOGY

FLII EFFECTS FLAGELLAR ASSEMBLY, MOTILITY AND BIOFILM FORMATION IN SALMONELLA TYPHIMURIUM

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Biofilms formation is a major hazardous problem from both clinical and environmental perspective. Flagellum-mediated motility is important for biofilm formation by several gram-negative bacteria. >50 genes are involved in flagellar biosynthesis and function in *Salmonella typhimurium*. The flagella basal body is a representative of Type III protein secretion systems; used by several gram-negative bacterial pathogens to colonize foreign tissues and substrates. The mechanism of flagellar assembly was analyzed in *S. typhimurium*, using bioinformatics analysis to identify conserved structural elements. In this study, *FliI* a flagellar protein that is needed for flagellar assembly and may be involved in a specialized protein export pathway was cloned and overexpressed. *FlhA* deleted mutant salmonella strain SJW1616 was used to transform *FliI* overproducing plasmid by electroporation. Using vital dyes (Alexafluor 488), visualization of motility was observed in wild type, SJW1616 ($\Delta flhA$) and *FlhA* transductant strain which was further assessed by biofilm formation ability. Swimming, swarming motility alongwith significantly reduced biofilm formation was observed in SJW1616 ($\Delta flhA$) compared to wild type and *FlhA* transductant strains. This study will extend initial evidence that *FliI* plays important role in flagellar export system and flagellum-mediated rotation is critical for swimming, swarming motility and biofilm formation. The flagellar basal body is a particularly convenient drug target, since the architecture of most its components has been determined near atomic resolution and it is an ancient evolutionarily conserved macromolecular assembly. The knowledge gained will also have implications for elucidation of the mechanistic design principles underlying protein secretion complexes.

AN OUTBREAK OF HUMAN BRUCELLOSIS AMONG GENERAL POPULATION OF FIVE SOUTHERN DISTRICT OF KHYBER PAKTUNKHWA, PAKISTAN

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Brucellosis is the zoonotic disease caused by bacteria of genus *Brucella* characterized by sweating, headache, weight loss, fever and joint pain enduring for week to months. The present study was conducted to determine the epidemiology of brucellosis in general population of Southern Khyber Paktunkhwa (KPK), Pakistan. 900 blood samples were collected from peoples of both rural and urban area of KPK. Serum samples were tested for presence of anti-*Brucella* antibodies by Serum Agglutination Test (SAT) and Rose Bengal Plate Test (RBPT). Overall incidence rate for brucellosis was 220(24.44%) and 180(20%) at RBPT and SAT respectively. Along the age positivity rate increases and highest rate was found in the age group of 46 and above years at both RBPT and Sat test. Similarly people of rural area showed high positivity at both RBPT (27.02%) and SAT (21.17%) test. Illiterate were at high risk as compare to educated ones, similarly people of low economic status showed high positivity rate at both test. Clinical feature like fever showed high positivity for brucella infection. People using raw milk and butter were more susceptible for brucellosis. It can be inferred that brucellosis infection continues to be a public health alarm and need good applicable strategy to control and eradicate the disease in the area.

SEROPREVALENCE OF *TOXOPLASMA GONDII* AMONG FEMALE PSYCHIATRIC PATIENTS OF LAHORE, PAKISTAN

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Toxoplasmosis is a cosmopolitan zoonosis, caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii*. It is worldwide in distribution. The unusual and abnormal behavior of psychiatric patients increases the risks of getting the infection of *Toxoplasma gondii*. The present study was conducted to find out the seroprevalence of *Toxoplasma gondii* among female psychiatric patients in Lahore, Pakistan. Blood samples were taken from 60 female psychiatric patients while blood samples of 60 non-psychiatric females were collected too in order to make comparison by using sterilized syringes. After collection of blood samples, serum separation was conducted by centrifuging the blood samples. The data regarding the gender, age, weight, socioeconomic status, educational level, occupation and presence or absence of cat as pet animal was recorded with the help of especially designed questionnaire. ELISA technique was used to determine the index of IgG *Toxoplasma gondii* antibodies. Data was evaluated on the basis of results. The results showed that percentage seroprevalence of *Toxoplasma gondii* in psychiatric females (case subjects) was 53.3% while in non-psychiatric females (control subjects) was 28.3%. Among psychiatric patients, the % seroprevalence was highest in schizophrenic patients (52.9%) while aggressive patients have least % seroprevalence (42.8%). The results suggested that risk factors such as contact with cat, unhygienic conditions are responsible for the transmission of infection. Other factors include unawareness, low socioeconomic status, and illiteracy rate play an important role in the transmission of infection. The results highlight the importance of sanitary education in the prevention of toxoplasmosis.

ANTIBACTERIAL EFFICACY OF CHITOSAN BASED IMMOBILIZED AND NON-IMMOBILIZED PROBIOTIC STRAINS AND THEIR ENZYMES ISOLATED FROM MILK

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Present study was deliberated to examine the antibacterial efficacy of probiotics and their enzymes against clinical pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* in both chitosan immobilized and non-immobilized form. *Lactococcus lactis* (A) and *Lactobacillus curvatus* (B), and their enzymes isolated (A1, B1, IA1, IB1, A2, B2, IA2, IB2, A3, B3, IA3 and IB3) and used for antibacterial action in both non-immobilized and immobilized form. It was observed that immobilized isolates showed lower zone of inhibition in particular hours with respect to non-immobilized probiotic. Results revealed that A1 was exhibited highly significant zone of inhibition against pathogens between the range 20.67 ± 0.47 mm to 28.67 ± 0.47 mm. On the other hand, *S. aureus* was strongly reduced by B1: 27.33 ± 0.47 mm, IB1: 16.67 ± 0.47 mm, B2: 26.33 ± 0.47 mm, IB2: 16.33 ± 0.47 mm and B3: 24.33 ± 0.47 mm, but moderate reduction was shown by IB3: 9.33 ± 0.47 mm. similarly, *E. coli* was highly inhibited by B1: 24.67 ± 0.47 mm, IB1: 17.67 ± 0.47 mm, B2: 26.67 ± 0.47 mm and IB2: 16.33 ± 0.47 mm, while no significant reduction by B3: 0.0 ± 0.0 mm and IB3: 0.0 ± 0.0 mm. *P. aeruginosa* was strongly inhibited (B1: 24.33 ± 0.47 mm, B2: 24.67 ± 0.47 mm and IB2: 13.67 ± 0.47 mm), moderately inhibited (IB1: 6.67 ± 0.47 mm), less sensitive (B3: 3.67 ± 0.47 mm) and no sensitive to (IB3: 0.0 ± 0.0 mm). *S. marcescens* growth was highly significantly decreased by B1: 27 ± 0.0 mm; B2: 26.33 ± 0.47 mm, moderately reduced by IB1: 7.33 ± 0.47 mm; IB3: 7.33 ± 0.47 mm and non-sensitive to B3: 0.0 ± 0.0 mm; IB3: 0.0 ± 0.0 mm. *K. pneumoniae* zone of inhibition was for B1: 26.67 ± 0.47 mm, IB1: 13.33 ± 0.47 mm, B2: 26.67 ± 0.47 mm and B3: 11.33 ± 0.47 mm, while moderate reduction was examined by IB2: 8.67 ± 0.47 mm and IB3: 8.33 ± 0.47 mm. *S. pyogenes* was highly sensitive to B1: 23.33 ± 0.47 mm, IB2: 16.67 ± 0.47 mm and B3: 26.67 ± 0.47 mm, moderately sensitive to IB2: 8.67 ± 0.47 mm and B3: 5.33 ± 0.47 mm, no sensitive to IB3: 0.0 ± 0.0 mm. *S. epidermidis*

was highly sensitive to B1: 25.33 ± 0.47 mm, B2: 22.67 ± 0.47 mm, IB2: 16.67 ± 0.47 mm and B3: 22.33 ± 0.47 mm, moderately sensitive to IB1: 7.33 ± 0.47 mm and IB3: 9.33 ± 0.47 mm. Findings indicates that the immobilized isolates were protected by chitosan coating material and showed less antibacterial effect. Hence, it was concluded that non-immobilized probiotics could be used as potent antibacterial agent against infectious pathogens.

IN VITRO SUSCEPTIBILITY OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM ACUTE AND CHRONIC PULMONARY INFECTION TO ANTIBIOTICS, *LACTOBACILLUS* COMPETITION AND METAL NANOPARTICLES

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Antibiotic resistance in *Pseudomonas aeruginosa* is a major barrier to successful treatment of infection. Additional and novel measures to control this pathogen are needed, along with contemporary information about antibiotic resistances that are present in isolates from different environments. In the present study 72 samples from blood, 43 from sputum, and 19 were obtained from tracheal aspirates patients suffering from chronic and acute lung infections admitted to a local hospital in Lahore. Susceptibility of 134 isolates of *P. aeruginosa* was tested against selected antibiotics (meropenem, imipenem, piperacillin, amoxicillin, amikacin, gentamicin, tobramycin, kanamycin, clarithromycin, cefepime, cefixime, levofloxacin, and ciprofloxacin), *Lactobacillus* strains and metal nanoparticles (copper, ferric and zinc). *P. aeruginosa* isolates showed *in vitro* resistance against 11 of 14 antibiotics tested. The isolates were highly susceptible to meropenem, piperacillin, and amoxicillin. It was also observed that the growth of these resistant *P. aeruginosa* strains was significantly inhibited in the presence of *Lactobacilli* spp. and nanoparticles of silver, zinc and ferric oxide at a concentration of 12, 200 and $1 \mu\text{g/ml}$, respectively. This study may help in the development of chemotherapeutic methods against multidrug resistant bacterial pathogens in chronic and acute lung infections. It provides a practical approach towards the use of nanoparticles to enhance antimicrobial activity against these pathogens.

PROCESS OPTIMIZATION OF AMYLASE PRODUCING BACTERIA USING RESPONSE SURFACE METHODOLOGY

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The aim of present study was to screen the amylase producing strains which had been previously isolated from fish gut. Moreover, the objective was also to optimize the production of amylase from *Bacillus cereus* in a medium by employing Response Surface Methodology. Eight strains of bacteria were revived on nutrient agar and on the basis of clear zones on selected medium, five strains were selected. Enzyme assay was performed for these strains, and on the basis of highest enzyme value ($177 \pm 0.00 \text{ U/ml}$), *B. cereus* was selected. The optimum temperature for *B. cereus* was found to be 37°C ($2.5 \pm 0.11 \text{ U/ml}$) as compared to 30°C and 45°C . Similarly, the optimum pH was found to be 9 ($20.48 \pm 0.20 \text{ U/ml}$) in comparison to pH 5 and 7. The 1% inoculum size showed highest enzyme activity ($12.87 \pm 0.25 \text{ U/ml}$) as compared to 3% and 5%. When incubation period was optimized, it was observed that 24 hours were an optimum condition for maximum ($22.48 \pm 0.12 \text{ U/ml}$) amylase production in comparison with 48, 72 and 96 hours of incubation. The medium components (starch, NaCl, CaCl_2 , K_2HPO_4 , MgSO_4 , and yeast extract) were screened employing 12 runs Plackett-Burman Design. From 12 experimental runs, 3 significantly affecting parameters (starch, MgSO_4 and yeast extract) were selected. For obtaining the mutual interaction between these variables and optimizing these variables, Box-Behnken Design (15 experimental runs) was employed and the combined effect of starch (0.5%), MgSO_4 (0.1%) and yeast extract (0.275%) showed the highest amylase production. The statistical model dictated the significance ($P < 0.001$) of model. The R^2 value was recorded up to 0.90. In present

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

OPTIMIZATION OF *BACILLUS SAFENSIS* FOR PROTEASE PRODUCTION USING RESPONSE SURFACE METHODOLOGY

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

IDENTIFICATION OF GIARDIA IN THE DIFFERENT DRINKING WATER SOURCES OF DISTRICT KARAK, KHYBER PAKHTUNKHWA, PAKISTAN

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A total of 400 water samples collected from five different sources (Tap, Pond, Dam, Hand pump and Well water) in district Karak of KHYBER PAKHTUNKHWA Province of Pakistan. In all the five sources, water was contaminated with *Giardia* parasite. The result indicates overall prevalence of 14.75% (59/400) of *Giardia*, including Tap water 23.75% (19/80), Pond water 21.25% (17/80), Dam water 17.5% (14/80), Hand pump water 05% (5/80) and Well water 6.25% (4/80). The result of the study presents a need of an appropriate source of drinking water to identify the threshold of water sources contamination that requires treatment. Preventing waterborne disease and the health effects of water contamination is vital to our nation's public health due to the fact that access to safe drinking water is required cornerstone of public health.

PREVALENCE OF *TOXOPLASMA GONDII* IN HUMAN FEMALES IN SWAT, PAKISTAN

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Toxoplasma gondii is an obligate intracellular, parasitic protozoon causes toxoplasmosis. Toxoplasmosis is widely prevalent all over the world, infecting most of warm-blooded animals including human. Serological studies

estimate that 30-50% of the global human population is infected with *T. gondii*. Toxoplasmosis causes serious complications such as stillbirth, abortion, different problems like mental and physical retardation, blindness and hydrocephalus. In present study total 216 blood samples were collected from symptomatic females from different localities of District. For the detection of parasite, lateral flow immune-chromatographic assay strip was used. Overall prevalence rate was 25.92%. The highest prevalence(33.33%) was recorded in age group 31-40 years. Lowest prevalence(18.6%) and (18.75%) were observed in age groups 11-20 and 41-50 respectively. Tehsil Bahrain was more effected (47.72%) while lowest prevalence(18.6%) was observed in Tehsil Kabal. Lower class families had high prevalence rate(34.32%) than upper class families (13.79%). Women using spring water were more affected(47.36%). Highest prevalence (43.93%) was observed in the females who had cats in their homes. 28% abortions and 21% still births were also observed in pregnant women. Special control measures and awareness in people is necessary, to reduce further spreading of toxoplasmosis in the area.

EVALUATION OF *EMBLICA OFFICINALIS* EXTRACTS FOR *IN VITRO* ANTIBACTERIAL ACTIVITY AGAINST EMERGING ANTIBIOTIC RESISTANT STRAINS

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The study was planned to assess the antibacterial activity of *Embllica officinalis*'s fruit against five bacterial strains: *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus coagulans* and *Escherichia coli* that are becoming increasingly resistant to antibiotics. Ethanolic and aqueous extracts were prepared by dissolving absolute ethanol and distilled water respectively in dried and ground fruit powder in 1:4 ratio (wt/vol). Filtration was done after cooling the solution followed by evaporation. Different dilutions were prepared (1%, 3%, 5%, 10%, 20%, 30%, 40% and 50%) and disc diffusion method was used to screen the antibacterial potential of extracts. Ethanolic extract of *Embllica officinalis* showed highest antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (21.84mm and 21.77mm) and lowest against *Bacillus coagulans* (19.64mm). While in case of aqueous extract the highest activity was found against *Pseudomonas aeruginosa* (25.17mm) and lowest against *Bacillus cereus* (19.50mm). Growth inhibition zones indicated that antibacterial activities were dose dependent and were found responsive to increase concentration of extracts. Comparative analysis revealed that the response rate was same at lower as well as at higher concentrations in both extracts ($P < 0.05$). Owing to its highly efficient antibacterial activity, *Embllica officinalis* hold immense potential for application in the treatment of infectious diseases.

DETECTION OF OPPORTUNISTS FREE LIVING AMOEBAE FROM DRINKING AND HOUSEHOLD WATER RESOURCES OF LAHORE

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Free-living amoebae are the opportunistic pathogens of human and other animals. They cause severe diseases such as encephalitis, keratitis and skin lesions in human and animal population. FLA include medically significant genera like *Acanthamoeba*, *Hartmannella*, *Balamuthia* and *Sappinia*. Overall, 59 water samples (13 filtered and 46 tap samples) were collected from different areas of Lahore. Samples were cultured for detection of FLA using non-nutrient agar (NNA). The isolates of free-living amoebae were initially identified on the morphological basis. Morphological identification showed flat-shaped trophozoites with discrete granules and spine like structure of acanthopodia and their size of ranges from 25µm-30µm. They have double walled cyst. Outer layer is smooth and inner layer is irregular. Cyst size measured 10 to 20µm were observed. Monopodial morphotype was observed in

samples of Chauburji and Islampura. Trophozoites were elongated having single nucleus and contractile vacuoles in cytoplasm. Locomotion of trophozoites was slow. Cysts shape was rounded having single wall. Cysts differed in size and shape. Size ranging from 11µm to 16.1µm. Eruptive form was present in Bund road water sample and all other characteristics were similar to monopodial morphotype. Furthermore, thermotolerance and viability assays showed that *Acanthamoeba* cysts were viable up to 70°C. Cysts were more resistant to chlorine and hydrogen peroxide disinfection. However, higher concentration of chlorine successfully killed both trophozoites and cysts.

CHARACTERIZATION OF ANTIBIOTIC RESISTANT CARBAPENAMASE FROM *ACINETOBACTER BAUMANNII*

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Acinetobacter baumannii are aerobic, non-motile and coccobacilli well known for their ability to develop resistance against antibiotics. They are responsible for causing colonization and infections in immunocompromised persons and patients that are present in hospitals and intensive care units. In WHO recommendations it is at top in the list of bacteria requiring new antibiotics. In present study we studied one environmental isolate and two clinical isolates of *Acinetobacter baumannii*. Biochemical characterization of these isolates showed they are catalase positive, gram negative, non-endospore forming, non-motile, obligate aerobes. These isolates showed maximum growth at 45 °C. Growth curves for these isolates were determined on optimum temperature. Antibiotic resistance against various antibiotics was detected by using disk diffusion method. This showed that the clinical isolates were more resistant to different classes of antibiotics as compared to the environmental isolate. The environmental isolate (1005) was susceptible to all the antibiotics except ampicillin and tetracycline antibiotics. The clinical isolate-3242 was resistant to all antibiotics except polymyxin B antibiotic whereas clinical isolate-3342Y was susceptible polymyxin B and imipenem. In *A. baumannii*, *bla_{OXA-51}* gene is most frequently found OXA carbapenemase. Environmental and clinical isolates were tested for the presence of this gene by PCR in which a 353 bp fragment of *bla_{OXA-51}* was amplified and then sequencing of amplified product. This gene was present in both environmental and clinical isolates.

WOLBACHIA INDUCED DENSITY AND LOCALIZATION PATTERN IN VARIOUS SOMATIC TISSUES OF *AE. AEGYPTI* FEMALES

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Naturally infecting bacteria, *Wolbachia* is transmitted maternally, present in more than 65% of insect species. *Wolbachia* induces pathogen blocking and rapidly spreads into mosquito vectors due to its potential of altering host populations through various mechanisms. These two characters have made possible to use *Wolbachia* in the control of vector borne diseases as a major biological control agent. Based upon the strain of *Wolbachia*, density of *Wolbachia* often varies under field conditions, which can potentially affect the transmission of virus, fitness and expression of cytoplasmic incompatibility CI (high-low) in insect host. Therefore, it is important to find density variation in different somatic and reproductive tissues of host vector to find a correlation between density and CI, virus inhibition and vector fitness etc. In the current study, *Wolbachia* density was compared among somatic tissues of transfected *Ae. aegypti* females. Somatic tissues (salivary glands, head, wings, fat bodies, midgut, and legs) from 6-7 days old female mosquitoes were taken and *Wolbachia* was confirmed through PCR. The positive samples were quantified using qPCR to find the density of *Wolbachia* in each tissue. All of the above-mentioned tissues were also dissected from laboratory colony of *Ae. aegypti* females without *Wolbachia*, served as control. The density in each tissue was calculated based upon the ratio of *wsp* (*Wolbachia* surface protein) and *rps6* (mosquito housekeeping) gene density. ANOVA indicated a significant higher *Wolbachia* density ($p < 0.001$) in salivary gland than other

tissues. Head region had one fold less *Wolbachia* density comparing with salivary glands and fat bodies. No significant difference in *Wolbachia* density was observed ($p>0.05$) between midgut and legs of *Ae. aegypti* females. The current results indicated the potential usefulness of *Wolbachia* based control strategies for vector population suppression and replacement that could provide important implications for the future vector-borne disease control.

ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SULFONAMIDE DERIVATIVES WITH PYRAZOLO TETRAZOLO TRIAZINE CORE AGAINST *ENTEROCOCCUS FAECALIS* AND *ENTEROCOCCUS FAECIUM*

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Enterococci are gram positive bacteria that are part of the normal intestinal flora of most the humans. In the last two decades, several reports have documented that the two most important species, *Enterococcus faecalis* and *Enterococcus faecium*, are among the leading cause of several human infections, including bacteremia, septicemia, endocarditis, urinary tract infections, wound infections, neonatal sepsis and meningitis. Bacterial infections and their increasing resistance to common antibiotics is a severe threat to global public health. To this end, synthesis of new drugs and testing their antibacterial efficacy is always desirable to meet unmet medical needs. In present study, sulfonamide derivatives (MM26-MM35) were synthesized and tested for their antimicrobial potential using clinically isolated *E. faecium* and *E. faecalis* using disc diffusion method. The result confirmed that the *E. faecium* showed dose depended inhibitory effect of all tested sulfonamide derivatives except MM32 and MM33. However, *E. faecalis* showed susceptibility to all tested sulfonamide derivatives except MM28, MM29, MM32 and MM33. Moreover, the compound MM25, MM34, MM28, MM26, MM27, MM31, MM35, MM30 and MM30 showed 0.71cm, 0.68cm, 0.63cm, 0.57cm, 0.53cm, 0.53cm, 0.53cm, 0.52cm and 0.45cm *E. faecium* inhibition, respectively at highest tested concentration (0.96mg/60µl). However, *E. faecalis* showed 0.7cm, 0.68cm, 0.57cm, 0.57cm, 0.55cm, 0.53cm and 0.52cm by MM35, MM25, MM34, MM27, MM31, MM30 and MM26 at highest tested concentration (0.96mg/60µl). Thus, effective sulfonamide derivatives inhibited *E. faecalis* and *E. faecium* successfully which may prove an effective alternative to treat bacteria in future.

ISOLATION, IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *SALMONELLA* SPECIES ISOLATED FROM POULTRY SAMPLES OF DISTRICT KOHAT

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Salmonella is comprised of a large circular chromosome consisting of approx. 4.8 mega bases (Mb). Extra-chromosomal DNA can be present in form of plasmids having various sizes. Until now 26 whole genomic *Salmonella* sequences are available. Essential proteins involved in metabolic pathways are 14 while 8 metabolic pathways are found to be present exclusively in the pathogen comprising of 27 enzymes unique to the pathogen. Thus, these 27 proteins may serve as prospective drug targets. *Salmonella* generates type 1 fimbriae, the most widely used type of fimbrial mechanism. Type 1 fimbriae expressed by serovar *typhimurium* were shown to cause persistent infection in swine. A total of 100 poultry samples were collected from different areas of District Kohat. The samples were collected from four major areas of district Kohat that include highway bypass, college town, Kohat main city and Kohat Development Authority (KDA). Samples were aseptically cut, tied and placed in sterile plastic bags which were kept on ice in an insulated cooler box and then transported to the laboratory and were processed on the same day. The samples were first washed with sterile distilled water and then surface sterilized with 3 % bleach, cut and chopped with sterile blades, then each sample was inoculated into tetrathionate broth at a ratio of 1:9 i.e. one gram of different poultry samples were inoculated into 9 ml tetrathionate broth. All samples were incubated at 37°C for 24 hours. The samples from the enrichment broth were then plated on bismuth sulphite agar for the isolation and preliminary identification

of *Salmonella species*. In this medium freshly precipitated bismuth sulphite acts together with brilliant green as a selective agent by suppressing the growth of coliforms, whilst permitting the growth of *Salmonella*. Sulphur compounds provide a substrate for hydrogen sulphide production, whilst the metallic salts in the medium stain the colony and surrounding medium black or brown in the presence of hydrogen sulphide. The plates were then incubated at 37 °C for 48 hours. On bismuth sulphite agar the plates having black color colonies were suspected to be positive while colonies of other color were considered as negative. A total of 100 poultry samples were processed in our research work, including different parts (Heart, Kidney, Liver, Chest and leg piece) of poultry. The overall prevalence of *Salmonella species* in these samples were 35 % (35/100). In heart the prevalence was 25 % (5/20), in kidney and liver the ratio of *Salmonella species* was 30 % (6/20) and 35 % (7/20) respectively. In chicken leg piece the high prevalence (45 %) (9/20) was noticed, While In chicken chest piece the prevalence of *Salmonella species* was 40 % (8/20). The prevalence of *Salmonella species* was more in Highway bypass and Kohat main city 44 % (11/25) and 40 % (10/25) respectively, in college town area of kohat the ratio of *Salmonella species* was 36 % (9/25), while the lowest prevalence (24 %) (6/25) of *Salmonella species* in poultry samples were observed in KDA. This study confirms the high prevalence (35%) of *Salmonella species* in the poultry samples and this study also confirm the high prevalence of antibiotic resistance of *Salmonella species* isolated from poultry samples of District Kohat. The antibiotic resistance profile indicates the limited therapeutic value of different antibiotics including, Ampicillin, Tetracycline, Ciprofloxacin, Levofloxacin, Azithromycin and Chloramphenicol. There is a need for continued surveillance is emphasized to determine regular antimicrobial susceptibility data to identify the changing pattern of resistance. Keeping in view the several possibilities of *Salmonella* contamination in the poultry industry, specific epidemiological studies on the spread of *Salmonella* at various levels of production is needed on a longterm basis.

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *PENICILLIUM* SPECIES ISOLATED FROM SILVER CARP, *HYPOPHthalmichthys molitrix* REARED IN EARTHEN PONDS

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This study was aimed to investigate morphological and molecular characteristics of *Penicillium* species isolated from silver carp. The fishes were obtained from three locations of Punjab. The mean total length was 18.32cm and mean body weight was 65.41g. Infected fishes had deep reddish gills, eroded scales, damaged caudal and pelvic fins, hemorrhages and lesions on the body. *Pencillium* isolated from infected parts (gills, skin and fins) of the body of the fishes were cultured on four different media. The inoculated agar plates were incubated for 5–7days at 28–32°C. Grey and white colonies appeared on agar plates. Isolated *Pencillium* were confirmed by internal transcribed spacer sequencing which matched to other *Pencillium* sequences available in GenBank. Two *Pencillium* species were confirmed i.e., *Penecillium crustosum* and *Pencillium marnneffe*. Infection observed in silver carp probably occurred through the use of contaminated feed and unsuitable fish farm environment. Fish infection by pathogenic fungi reduces the economic and nutritional value of fish. The present situation points our attention for very good fish health management practices in the fish ponds.

SYNTHESIS OF SILVER NANO PARTICLES FROM CURRY, NEEM LEAVES, CITROBACTER AND CHECK ITS ANTIMICROBIAL ACTIVITY AGAINST STAPH AND PROTEUS

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Nanotechnology is the study and application of very small particles and may be used in different opposite fields namely biology, physics, chemistry, engineering and materials science. Applied science is quickly rising by producing the nanoparticles (NPs) which will have new and different physical and chemical properties and size from

higher matter. These new properties of NPs are exploited in a very large choice of probable uses in drugs, makeups, renewable energies, ecological modification and remedy devices. In between them, the silver nanoparticles (Ag-NPs) have fascinated growing interest because of their different biological and physiochemical properties. These are the silver nanoparticles of in between 1-100 nm of size. Nanotechnology is the study and application of very small particles and may be used in different opposite fields namely biology, physics, chemistry, engineering and materials science. The silver nanoparticles (Ag-NPs) have fascinated growing interest because of their different biological and physiochemical properties and in between 1-100 nm of size. First taken 10g of powder in 100 ml of distal water in conical flask. Cover the mouth of conical flask with cotton pluck. Set the temperature of water bath at 80 °C and after temperature reached 80 °C, kept the conical flask in water bath for 10 minutes. Filter the extract through whatman 1 filter paper. Collect the extract in separate sterile conical flask and stored the conical flask in refrigerator at 4 °C respectively. The same procedure was also repeated for Curry and Neem leaves. Taken 2.5 ml of ammonium solution in separate sterile conical flask. Added 10 ml plant extract and 32.5 ml deionized water in conical flask. After looked the solution color who changed from yellowish to brown indicated the presence of silver nano particles. Kept all solution in Erlenmeyer flask and incubated at 37 °C under agitation (200 rpm) for 24-48 hours. We have taken 2 samples of different plants made extract and synthesized the silver nano particles from Curry, Neem extract and microorganism. It also checked its frequency at different wavelengths, presented by various above graphs. The graphs indicated different wavelength with different frequencies. We also made silver nano particles from Citrobacter. It gives oxidase negative and citrate positive with mucoid colonies on Eosin-Methylene Blue. Brownish yellow color indicated the confirmation of silver nano particle production. We have shown to check the zone by yellow agar diffusion method and placed the erythromycin antibiotic which shows high antimicrobial activity against Proteus and small antimicrobial activity against Staph. Erythromycin is an antibiotic which inhibit protein synthesis and silver nano particles are also inhibit growth of Proteus which are synthesized from curry leave extract and microorganisms. We also shown the zone of silver nano particles who synthesized by Citrobacter. It is indicated that the silver nanoparticles who synthesized from Curry, Neem leaves extract and Citrobacter recorded high values with range of 350 - 450 wavelengths respectively. The current findings were concluded that the Curry, Neem leaves extract and Citrobacter play an important role inside the reduction and stabilization of silver to silver nanoparticles. It also showed medicinal activity on every gram positive and gram negative microorganism. Furthermore, it is utilized in several medicines and cosmetics etc, because it has conductive and optical properties. It has low priced, material free and eco-friendly.

MERCURY DETOXIFICATION ABILITY OF NITROGEN FIXING BACTERIAL STRAINS ISOLATED FROM DIFFERENT AREAS OF THE PUNJAB, PAKISTAN

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Presence of heavy metals such as mercury in the environment especially in water and soil poses toxic effects to plant growth and human health. Mercury being non-essential and toxic heavy metal adversely affects plant root and shoot formation, nutrient uptake ability, yield and starts accumulating in crops. The consumption of such plants results the entry of mercury in food chain leading to the serious health problems to human and other animals. In the present study, bacterial strains were isolated from Hg-contaminated soils collected from different areas of Punjab province of Pakistan. The isolated bacterial strains were screened out due to their resistance at 30 µg/ml of HgCl₂. Selected strains were also tested for their plant growth promoting abilities by using different tests such as phosphate solubilization, nitrogen fixation, HCN production and IAA production. Six bacterial strains showing high resistance against HgCl₂ and plant growth promoting abilities were identified by 16S rRNA gene sequencing as *Bacillus subtilis* (KJ736013), *B. pumilus* (KJ736015), *B. cereus* (KJ736012), *Pseudomonas aeruginosa* (KJ736016), *Enterobacter cloacae* (KJ736014) and *Exiguobacterium* sp. (KJ736011). The selected bacterial strains showing dual characteristics can be utilized not only for the remediation of mercury but also for the enhancement of plant growth. It is concluded that the selected bacterial strains can be used as biofertilizer in Hg-contaminated agricultural lands for making sustainable agriculture.

COMPARISON OF METHODS FOR DETECTION OF *CANDIDA* IN BRONCHO-ALVEOLAR LAVAGE OF CANCER PATIENTS

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An accurate and rapid identification of *Candida* species in cancer patients with pulmonary symptoms can provide important information for effective treatment. We used conventional methods such as culture, fermentation reactions, morphology while molecular methods based on the ribosomal DNA repetitive regions or the Internal Transcribed Spacer (ITS) for the identification of *Candida* species. Seventy bronchial specimens of Bronchoalveolar Lavage (BAL) from cancer patients at Shaikat Khanum Memorial Cancer Hospital & Research Center, Lahore, Pakistan were included in this study. Seventy cancer patients were diagnosed on the basis of histological profile. *Candida* detected by conventional methods using Sabouraud Dextrose Agar (SDA), potassium hydroxide (KOH) preparation, germ tube test, fermentation reactions and Gomori methanamine-silver stain (GMS). Thirty (42%) positive isolates of *Candida* species were obtained by culture, twenty (28%) isolates were germ tube test positive while thirty isolates (42%) were positive by PCR method. In conclusion, the results of our study showed that the PCR based detection methods are significantly better and can detect *Candida* with more accuracy and specificity as compared to conventional methods. Our study would pave the path for optimization of protocols for detection of *Candida* in cancer patients.

BACILLUS SUBTILIS AS BIOLOGICAL FACTORY; SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST MULTI-DRUG RESISTANT BACTERIA

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Silver nanoparticles holds promising potentials in therapeutics due to characteristic properties and multiple applications. Biological synthesis of Silver Nanoparticles (AgNPs) holds supremacy upon the physical and chemical synthesis which may pose serious toxicities. In this study, silver nanoparticles were synthesized from non-pathogenic *Bacillus* strain and its antimicrobial activity was assessed. One *Bacillus subtilis* strain (FCBP-WB-0174) was selected on the basis of its silver resistant nature among four strains, FCBP-SB-324, FCBP-SB-223 and FCBP-SB-0189. Different strains of *Bacillus* spp. were selected and screened against AgNO₃ toxicity. Silver nanoparticles were synthesized from culture supernatant of *Bacillus subtilis* at 37°C which was found resistant to AgNO₃ toxicity. The conditions were optimized and the silver ion reduction was found at a ratio 1:1 from all the three molar concentrations (1mM, 2mM and 3mM) of AgNO₃. Synthesized AgNPs were characterized by UV-Vis spectrophotometry and Scanning electron microscopy (SEM). The characterized nanoparticles were found to have a characteristic absorption peak at 426 nm and the particles were found to have spherical shape under SEM with an average diameter of about 80±0.18 nm which was also reconfirmed using Zeta Sizer Nano. These AgNPs were used for testing antimicrobial activity against five multi drug resistant bacterial strains. Prepared Silver nanoparticles have potential antimicrobial activities to all tested pathogenic strains e.g. *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Methicillin-resistant *Staphylococcus aureus* (MRSA). So, it was found that effective AgNP's were produced from selected *B. subtilis* strain and the strain itself was resistant to AgNO₃. These results proved biologically synthesized silver nanoparticles from *B. subtilis* has encouraging antimicrobial activities against pathogenic and multidrug resistant bacteria.

SURVEILLANCE OF RESISTANCE PROFILE AND RISK FACTORS ANALYSIS AMONG *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

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Pseudomonas aeruginosa is an important nosocomial pathogen, frequently associated with morbidity and mortality in immunocompromised patients due to the immuno-ablative treatments, neutropenia and prolonged hospitalization. The ability of *Pseudomonas aeruginosa* to survive in the healthcare setting makes it a frequent problematic pathogen. Much of the interest in *Pseudomonas aeruginosa* has been attributed to its remarkable rapid acquisition of resistance mechanisms. *Pseudomonas aeruginosa* is an excellent example of genetic plasticity, with its ability to acquire and express resistance in plasmids and chromosome particularly to aminoglycoside, carbapenem, fluoroquinolones and miscellaneous. The present study was undertaken to determine the prevalence of Multi drug resistance of *P. aeruginosa* that was isolated from Jinnah Hospital in Lahore. Determine the prevalence of resistance specifically against aminoglycoside, carbapenem and fluoroquinolones. In the conclusion of this study, we find prevalence, antibiotic resistance profiling in sputum, urine, tracheal secretions, and exudate specimens were processed by Gram dye technique, biochemical reactions and culture techniques by Kirby bauer disk diffusion method.

GENETIC AND METABOLIC CHARACTERIZATION OF *PSEUDOMONAS* SP. STRAINS EXHIBITING BIOFERTILIZER TRAITS AND BIOCONTROL POTENTIAL

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Fluorescent pseudomonads have widely been exploited for their use as biofertilizers and biocontrol agents and are considered as key players in sustainable agriculture. However, very few studies comprehensively characterize the putative biocontrol genes, antagonistic metabolites, and biofertilizer traits, simultaneously. This study evaluated four different species of *Pseudomonas* for their *in vitro* biocontrol potential against fungal phytopathogens, role in wheat growth promotion, and characterization of antimicrobial compounds. Molecular characterization of *Pseudomonas* sp. isolates was based on 16S rRNA gene while antifungal activity of these strains was determined by agar well diffusion method. Secondary metabolites were subjected to LC/ESI-MS/MS for confirmation of compounds and [M + H]⁺ ions were monitored. All strains were also screened for plant growth promoting traits and plant experiments were conducted on wheat in climate control room. On average, all strains exhibited 0.8 cm inhibition zones against the causal agents of root rot, seedling blight, red and stem rot. All eleven strains showed the production of six phenazine derivatives, 2-acetamidophenol, pyochelin, pyocyanin and *N*-acylhomoserine lactones while pyrrolnitrin, WLIP, 2-hydroxyphenazine and 2,8-dihydroxyphenazine were unique to *P. aurantiaca* strains only. This study demonstrated the production of three new ortho-dialkyl-substituted aromatic acids; Lahorenoic acid A, B, C and WLIP from *Pseudomonas chlororaphis*. IAA, HCN, protease and lipase production were observed by all strains while cellulase production, phosphate and zinc solubilization was variable. *P. aurantiaca* strains; ARS-38, RP-4, PB-St2 and *P. fluorescens* RS-1 considerably increased wheat shoots and roots biomass and lengths. These findings indicate the potential of these *Pseudomonas* strains to be used as user-friendly single-strain bioinoculums with multifaceted biocontrol and biofertilizer traits.

BIOCHEMICAL PROFILING AND ANTI-MICROBIAL ACTIVITY OF *THYMUS VULGARIS*

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Thymus vulgaris extracts were tested to check the biochemical and phytochemical profiling. The extracts of were separated on the basis of polarity of solvents like chloroform, n-hexane, butanol, methanol, ethyl acetate and distilled water. These extracts were utilized for phytochemical screening to break down the nearness of flavonoids, coumarine, quinone, tannins, terpenoids and saponins mixes. In the meantime, same extracts were also used to check their antifungal activity by deploying against different contagious strains like *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavos*. Spectrophotometer was used to survey the antioxidant activity of extracts which included POD, SOD, alpha-amylase and protease. Each enzymatic measure indicated distinctive absorbance at assorted wavelength (nm). Radical scavenging activity of *Thymus vulgaris* extracts were tested by utilizing DPPH compound at various fixations. At each concentration, *Thyme* extracts presented distinct activity. Phytochemical screening of *Thyme* extracts demonstrated that flavonoids, saponins, tannins, quinone were available in least way while terpenoids and coumarine were exceptionally identified in each weakened concentrate. While in antioxidant activity, it was observed that every enzyme (SOD, POD, alpha-amylase and protease) indicated distinctive action at various concentrations. Along these lines, distinctive absorbance demonstrated by *Thyme* extracts at their applicable wavelength. In DPPH radical scavenging activity, general outcomes showed that maximum activity exposed by diluted extract at 50 µL while least action showed at 100 µL. While if there should arise an occurrence of TPC evaluation, it was assessed that most elevated TPC was available in methanol extract while least TPC was uncovered in distilled water extract. The hemolytic action of *Thyme* extract showed that butanol extract indicated most extreme movement and ethyl acetate uncovered least action. The results of antibacterial activity showed that positive bacterial strains were more sensitive as compare to negative strains towards chloroform, n-hexane, butanol, methanol, ethyl acetate and distilled water extract.

PROBIOTIC DEVELOPMENT AND ITS APPLICATION IN PAKISTAN PERSPECTIVE

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Undoubtedly food safety is significantly import to customer, manufacturer and economy of every Country. Due to public awareness and eagerness for natural food products, alternative conventional techniques have been used. The development of probiotics provides a chance for the regulation of gut microbiota towards ensuring health. The main goal of using probiotics is to enhance immune system and to reduce stress tolerance level. Most commonly two bacterial species such as *Lactobacillus*, *Bifidobacterium* have been distinguished to employ beneficial effect on changing natural microbiota. However, its need of hour is that these exogenic species remain in pristine form when gain access to large intestine. The most common microbiota are used in many ways like restraint of pathogenic organism, diminished cholesterol level, fostering the immune system and trimming of inflammatory bowel disease. Different biostratagic therapies have been evolved. One of this is fecal microbiota transplant was successfully used to treat CDI, IBD and other diseases. Overall this study summarized information regarding to probiotics and their applications in Pakistan perspective. It focusses on how probiotics effect the natural microbiota on a beneficial way. Finally, number of products are reviewed regarding their utility, effect and safety concern. In future probiotic intervention can act as replacement of antibiotic to ensure long lasting animal and human health. This study also highlights the rules and regulations/acts regarding food quality and security prevailing in Pakistan.

MICROBIAL ANALYSIS AND SPIROMETRIC ASSESSMENT OF LUNG FUNCTION OF THE INHABITANTS OF UPPER HUNZA, GILGIT BALTISTAN, PAKISTAN

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Good Air quality is the driving force behind respiratory health, so the current study was aimed to assess the microbial contamination of air and respiratory health condition of the inhabitants of upper Hunza by microbial analysis of air and spirometric assessment of lung function. A questionnaire survey was also carried out, to find out the occupational load and its impacts on the health along with general environmental conditions of the study area. The study was conducted from October, 2016 to May, 2017 and data was taken for indoors as well as outdoors, for the summer and winter seasons. The Total Suspended Particles (TSP) were higher in indoors and outdoors during the winters (15.56 µg/L & 0.203 µg/L respectively) as compared to summers (1.267µg/L & 0.024 µg/L respectively). The number of participants for spirometry was 41, Mean pulmonary parameters showed the values for FVC (L); pred 2.98, PRE 2.64, % pred 89.49, FEV1 (L); Pred 2.50, PRE 2.35, %pred 94.63, FEV1% (%); Pred 81.83, PRE 89.65, % pred 109.93 and PEF (L/s); Pred 6.70, PRE 3.43, %pred 50.90. The oximetry results showed mean peripheral capillary oxygen saturation (SpO₂) value of 90.48 %. The average beats per minute (BPM) was 92.27. The various spirometric test results were compared on the basis of age, working hours, pesticide use and chest tightness. On the basis of the results it was concluded that, the indoors had higher number Colony Forming Units (CFU) and differential colony counts as compared to outdoors for both winter and summer, (64×10⁶ CFU/m³ and 36×10⁵ CFU/m³ respectively). Winters showed high CFU both indoors (64×10⁶ CFU/m³) and outdoors (9.6×10⁶ CFU/m³) as compared to summers indoors (3.6×10⁶ CFU/m³) and outdoors (1.4×10³ CFU/m³). Microbial identification showed the presence of 6 bacterial types, with the dominance of *Staphylococcus aureus* found in all four sites. Comparing the lung function test results, the working duration was found to have no significant effect on pulmonary function of local farmers. Age was found to be a significant effecting factor on SpO₂. Farmers who were complaining of chest tightness had significant difference in the FEV1 than those who had no chest tightness problem. Our study depicts that in winters the health of people was at more risk in terms of TSP and viable bacteria. The reasons may include poor living styles and inadequate air ventilation in winters as compared summers. The higher number of bacterial colony indoors highlights the favorable temperature for the growth of colony which should be addressed to make the indoor environment safer.

POTENTIAL USAGE OF HEAVY METAL TOLERANT BACTERIA IN BIOREMEDIATION AND IN AGRICULTURE

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Heavy metals pollution adversely affects soil microbial community and soil health. The present study was conducted to isolate bacterial strains tolerant to heavy metals (Cr, Cu, Pb, As, Cd) and to elucidate their potential usage in bioremediation and in agriculture. More than 68 strains were isolated from industrial discharge and screened for maximum tolerance limit (MTL) of heavy-metals. It was found that some strains showed a maximum tolerance up to 3600 ppm for Cr, 3300 ppm for Cu, 3000 ppm for Cd and As, 2100 ppm for Pb. Phylogenetically different strains

(23) were further analyzed for biosorption of heavy-metals. Maximum biosorption occurred for Pb followed by Cd and Cu, whereas biosorption of As and Cr was significantly lower by all the isolated strains. 16S rRNA gene sequence based molecular identification of heavy-metals tolerant strains demonstrated that the isolated strains belonged to 19 genera and majority of isolates were related to genera, *Bacillus* (21 %), *Pseudomonas* (12 %) and *Staphylococcus* (10 %). Based on the results of molecular characterization for *nifH* and *acdS* gene(s), at least 7 strains were found to contain both genes in their genome (15 isolates contained *nifH* gene, whereas 8 strains showed *acdS* gene). Four strains (NCCP-650^T, NCCP-644, NCCP-614, and NCCP-602) were investigated for plant growth promotion activity in *Brassica napus* under axenic condition when irrigated with water containing 50 ppm of each metal separately. The results showed that the strains increased growth of *Brassica* plants in comparison to control (no addition of strain or metal). Among the strains, NCCP-650^T proved to be the best for maximum increase in growth of *Brassica* plants. These heavy-metals tolerant strains may have potential for plant growth promotion and can be used as bioinoculants (biofertilizer) in agriculture of heavy-metals contaminated soils. Our results also indicated that some of these isolates can be used for bioremediation of soil/water system contaminated with heavy-metals such as Pb, Cd and Cu.

ISOLATION OF GRAM-NEGATIVE BACTERIA FROM COCKROACHES RECOVERED FROM HOSPITAL ENVIRONMENT AT QUETTA, BALOCHISTAN

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Cockroaches of the insect order Dictyoptera play a significant role in transmitting of various diseases either mechanically and biological because they harbor and spread various species of microorganism. This study was conducted in order to isolate the pathogenic bacteria from external and internal body of cockroaches from three different study areas. A total of 98 cockroaches, 43.7% of *Periplaneta americana*, 34.1% of *Blattella germanica* and 22.5% of *Blatta orientalis* were collected at hospitals premises viz civil hospital, Bolan medical complex hospital (BMC hospital) and Benazir hospital in Quetta metropolitan, Balochistan. Medically important bacteria were isolated from external and internal surface by using microbial technique. From the collected *Periplaneta americana*, *Blattella germanica* and *Blatta orientalis*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Salmonella* spp. and *Shigella* spp. were isolated from the gut and external surface and identified in their selective media such as, Eosin methyl blue (EMB) agar, MacConkey agar, and Cetrimide agar using microbial technique. The data obtained from the study emphasized that cockroaches may play an important role in the transmission of pathogenic bacteria to human. The presence of cockroaches at hospital units (wards, cafeteria, lawns) maybe due to unhygienic environment. Thus it may pose as a safety issue as they may carrier of pathogenic microorganisms that can threaten the health of individuals.

5. MOLECULAR BIOLOGY

DYSREGULATED EXPRESSION OF COLLAGENS IN DIABETIC FOOT ULCER

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Diabetic Foot Ulcer (DFU) is the most devastating complication of Diabetes Mellitus, pigeonholed by hyperglycemia and impaired wound healing. There are number of molecular and genetic components involved either directly or indirectly in its pathophysiology. Present study was conducted to analyse the mRNA expressions of collagens including *COL1A1* and *COL3A1*, which plays crucial role in extracellular matrix deposition. A total of 24 foot tissue samples were collected, 16 from DFU patients undergoing treatment and 8 from nondiabetic foot skin (NFS) of healthy controls. Samples were further processed for RNA extraction and Quantitative polymerase chain reactions (qPCR) were carried out to measure the gene expression of collagens in DFUs and NFS controls. Significant downregulation of *COL1A1* ($p=0.0001^{***}$) and *COL3A1* ($p=0.0192^{*}$) in DFU tissues has been observed in comparison to NFS at the mRNA level. There might exist strong association between reduced expressions of *COL1A1*, *COL3A1* and disrupted deposition of extracellular matrix during diabetic foot ulceration.

USE OF CO1 GENE SEQUENCES FOR COMPUTING GENETIC DIVERSITY BETWEEN *CIRRHINUS MRIGALA* FROM TWO DIFFERENT HABITATS (FARM AND RIVER)

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DNA barcoding technique involving identification of animal species based on a partial sequence of mitochondrial cytochrome C Oxidase 1 gene attracted many scientists at the beginning of 21st century. The flaws in traditional morphometric methods for identification of animals/fish larvae, eggs, and processed, damaged fish specimen upraised the question for an alternate method for identification and grouping of many animal species. Partial sequence of CO1 gene used as barcode has been proved a useful tool for identification of fish species as well as helpful in computing evolutionary history and genetic diversity. Current study was conducted to identify the fishes by using the DNA barcoding technique resulting partial sequences of CO1 gene, and then use of these sequences in evaluation of the evolutionary history and genetic diversity of *Cirrhinus mrigala* inhabiting different areas. Short sequences from 5' end of CO1 gene (650 base pair) were amplified, sequenced and analyzed using different soft wares. According to results haplotypes were found which showed genetic variations among fishes from two different habitats (farm and river). The phylogenetic analysis established a close relationship and a common ancestor of fishes from different habitats. Partial sequences of CO1 gene of all fish used in the study showed haplotype diversity of 0.7143. From these results it is concluded that fish species sharing same genus and family but different habitats show genetic diversity even though sharing a common ancestor.

ESTIMATION AND CORRELATION OF MALONDIALDEHYDE (MDA) AND BIOCHEMICAL RESPONSE IN PATIENTS OF OVARIAN CANCER

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Ovarian cancer shows highly heterogeneous nature. Ovarian cancer refers to any cancerous growth that occurs in the ovary. The majority of ovarian cancers arise from the epithelium (outer lining) of the ovary. Ovarian cancer remains the main cause of death from gynecological malignancy. The ovaries are two small organs located on either side of the uterus in a woman's body. 5.0 ml blood sample of 30 diagnosed ovarian cancer and 25 age and sex matched healthy individuals was taken from vein in clotted gel vials from oncology department of Mayo hospital and Jinnah Hospital Lahore. The estimation of MDA, CAT, GSH, SOD were estimated. The spectrophotometric reading of samples portrays that MDA level in ovarian cancer patients is remarkably inflated than normal person (8.42 ± 0.53). Whereas their level in healthy individual is extremely low (2.25 ± 0.24). The value of GSH demonstrates that in cancer patients the level of GSH reduced (0.12 ± 0.14) as compared to normal individual (2.14 ± 0.19). The CAT level is moderately decreased in patients than normal person (0.60 ± 0.52). The value of healthy individual is high (4.16 ± 1.06). Results parade the amount of SOD that is slackening in ovarian cancer patients (1.02 ± 0.02) though it is high in normal people (2.15 ± 0.25). Patients of ovarian cancer has remarkably deficient vitamin A than normal person (2.55 ± 0.17). Its level is extremely high in healthy subjects (7.15 ± 0.47). The outcome obtained revealed the worth of significant ($p=0.000$). There is also reduced level of Vitamin E in effected person (0.57 ± 0.27) than normal (4.33 ± 0.97). So it depicts that ovarian cancer patients shows the highly significant behavior ($p=0.000$). Antioxidant enzymes play a significant character in the defense of cells from oxidative damage. A fine balance between oxidants (ROS production) and antioxidants is predominant for the general protective capacity of cells in contrast to oxidative damage. The antioxidant enzymes, SOD and CAT, extensively dispersed in all cells, are commenced in large quantity in erythrocytes. It was presumed that ovarian cancer was an asymptomatic disease. We remarked that 95% of women with ovarian cancer described symptoms prior to investigation.

A MODIFIED *IN VITRO* TRANSCRIPTION APPROACH TO IMPROVE RNA SYNTHESIS

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RNA elements such as catalytic RNA, riboswitch, microRNA, and long non-coding RNA (lncRNA) perform a major role in cellular processes. A complete understanding of cellular processes isn't possible without knowing the structure-function relationship of participating RNA molecules that ultimately requires large quantities of pure RNA. Thus, structural/functional analyses of emerging RNAs necessitate revised protocols for improved RNA quantity and quality. Here we present an optimized *in vitro* transcription protocol to enhance RNA yield and ribozyme cleaving efficiency by working on two variables, i.e. incubation temperature and limiting GTPs. RNA from transcription mixture is subsequently purified through denaturing size-exclusion chromatography (SEC). We observed that cyclic elevated temperatures following low-temperature incubation during transcription elongation and an increased concentration of GTPs improve an overall yield of RNA. Our modified *in vitro* transcription method improves the ribozyme cleaving efficiency and target RNA yield by four-fivefold that can benefit almost any RNA-related study from protein-RNA interaction analysis to crystallography.

DETERMINATION OF POSTMORTEM INTERVAL BY ESTIMATING CHANGES IN MRNA LEVEL OF B-2-MICROGLOBULIN GENE IN MOUSE FOR FORENSIC APPLICATION

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Postmortem interval (PMI) is the time period elapsed between the death of an organism and the beginning of an

official death investigation. Reliable estimation of PMI is crucial in the field of forensic science for the investigation of criminal cases involving homicide. Time dependent change in mRNA level provides a valuable tool for reliable estimation of PMI in addition to conventional markers for PMI determination. Present study investigated the correlation of total RNA concentration and *B2M* mRNA level with increasing PMI. RNA was extracted from liver tissues of 10 mice (5 male and 5 female) after incubation at 4°C and 37°C for 0, 2, 4, 7, 14, 21 and 28 days postmortem. *B2M* mRNA expression level was estimated by reverse transcriptase Real-Time PCR analysis. Mean, standard deviation and correlation coefficient was calculated. Statistical analysis showed a gradual and almost linear decline trend of total RNA concentration and *B2M* mRNA level with increasing PMI at both incubation temperatures but with a higher rate of decline at 37°C. It was observed that total RNA concentration has negative and Ct-value has a positive Pearson's correlation with PMI whereas *B2M* mRNA is negatively correlated with increasing PMI. It was concluded that *B2M* gene can be a PMI prediction marker within 3 to 21 days at 4°C and within 21 days at 37°C. Moreover, PMI prediction potential of *B2M* gene was found to be 93% accurate by estimating Ct-values of test samples with unknown PMI from average Ct-value graphs of experimental samples with specified PMI in days. Time dependent change in mRNA level of *B2M* gene after death can be a complementary tool to traditional parameters with the ultimate goal to increase the accuracy and reliability of PMI estimation and can be a part of forensic testing after conducting similar studies on human subjects for its application in death investigation.

MICROBIAL FORENSICS VIA GERM PLASM EXCHANGE: THE NEXT FORENSIC CHALLENGE

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A new branch of forensics "microbial forensics" has been shaped, which focus on depiction of evidence from a bioterrorism to bio crime. With a threat of bio crime, the pathogens and toxins which could be converted into bioweapons got importance. Here we are presenting the pioneer research work, started in Centre in Excellence in Molecular Biology on forensic microbiology. Mode of investigation is principally the same as any other forensic investigation concerning processing *i.e.* scene investigation, chain of custody practices, evidence collection, handling and preservation, evidence shipping, analysis of evidence, interpretation of results and presentation. The use of germ plasm exchange as a tool of bioterrorism was investigated by analyzing the genetic diversity of *Xanthomonas oryzae*, pv. *Oryzae*, (a major pathogen of rice crop), as a test case. Primers complementary to sequence in IS1112, a repetitive element isolated from *Xanthomonas oryzae*, pv. *oryzae*, were used to fingerprint DNA from 200 bacterial blight isolates. Out of these 200 isolates 22 were found highly polymorphic. Similarity index and analysis through UPGMA confirms the exchange of germ plasm from source to spot.

CLONING AND ANALYSIS OF MC1R GENE IN NUBIAN GOATS

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In recent years, melanin enriched dynamic plant food favored to explore the molecular mechanism for the formation black coat color in Nubian goats. This study is characterized to analyze biological information and melanin cortical prime receptor -1MC1R (melanocortin 1 receptor) in the cloning of black, brown and grey Nubian goats. Primer were designed according to the sequence published in NCBI (GI:106503093) for goat MC1R gene and is used as template to extract the genomic DNA sequence from blood samples of the Nubian goat. Cloned gene was amplified through PCR that consist of 954bp. The results of multiple sequence comparison for cloned fragment represents similarity with that of pig, cattle, human, dog, sheep, mouse and chicken, were 99%, 87%, 86%, 85%, 84%, 81% and 77%, respectively, showing that the MC1R gene is highly conserve in different

species. Nubian goats MC1R protein having molecular mass of 34.65 Ku, isoelectric point (IPE) 8.70, weakly alkaline, containing the membrane receptor proteins typically with seven trans membrane domains. Total three SNP loci were detected; one base pair was deleted and other two were abnormal and also showed three different colors in Nubian goats at MC1R coding regions. The spouted out results may be used as a significance reference for further study of MC1R gene for Nubian goat hair color, genetic mechanism, gene mapping and its gene expression regulation.

PCR AND CLONING OF RECOMBINANT GROWTH HORMONE CDNA FROM CHOLISTANI COW

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Cholistani cow breed is a zebu (*Bos indicus*) or one humped breed of cattle being reared by the nomadic pastoralists of Cholistan desert, Pakistan. With an increasing human population and improving living standards, the demand for meat and milk is also increasing. To cope with such conditions in Pakistan especially in Cholistan, recombinant technology is used. Importance of recombinant growth hormone rGH in milk and meat production bovine growth hormone bGH has been cloned from a number of species like buffalo, sheep, goat, sheep, and horse etc. Present study was carried out to clone bGH from Cholistani cow. For this purpose, total cellular RNA was extracted from the pituitary gland of freshly slaughtered animal, cDNAs were synthesized and amplified with the help of sequence specific primers. The amplified products were confirmed through restriction digestion and finally cloned in Thyamine Adenine T/A cloning vector followed by transformation in *E.coli* strain DH5a. Positive clones were confirmed by restriction digestion and sequence analysis confirmed clones were stored at -70°C as glycerol stock.

EFFECT OF UPSTREAM MODIFICATIONS ON EXPRESSION OF CRY2AC11 IN *BACILLUS THURINGIENSIS*

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Cry2Ac11, a 65 kDa insecticidal protein produced by *Bacillus thuringiensis*, shows toxicity against dipteran and lepidopteran larvae. It is encoded by *cry2Ac11* gene (*orf3*), which is part of an operon comprising *orf1*, *orf2*, and *orf3*. Orf2, a helper protein, helps in proper folding and prevents aberrant aggregation of newly produced molecules. In this study, we have elucidated the effect of different mutations in translation initiation region (TIR), particularly the ribosomal binding site and the start codon (RBS-ATG) on *cry2Ac11* gene expression without helper protein. All recombinant constructs were expressed in acrySTALLIFEROUS *B. thuringiensis* subsp *israelensis* 4Q7 under the control of strong chimeric promoter *cytIAP/STAB*. Of all the mutants, mut/RBS2, with two consecutive AUGs after the spacer region in TIR, exhibited 89- and 2246-fold higher transcript levels compared with 4Q7-operSalI/RBS (*cry2Ac11* operon) and 4Q7-w-RBS (*cry2Ac11* gene), respectively. The analysis of *mut/RBS2* messenger RNA (mRNA) structure in the RBS-AUG region showed the presence of RBS in the single-stranded part of the moderately stable hairpin loop. The high expression efficiency of Cry2Ac11 mutant without helper protein is a cumulative and cooperative result of chimeric promoter *cytIAP/STAB-SD* with the optimal context of RBSAUG region provided by multiple AUGs and stabilizer sequence at 3' ends.

COMPARATIVE INSECTICIDAL ACTIVITY OF CRY2AC AND CRY2AD TYPE TOXINS OF *BACILLUS THURINGIENSIS*

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Cry2A toxin of *Bacillus thuringiensis* (Bt) shows toxicity against dipteran and lepidopteran larvae. The *cry2Ac* and *cry2Ad* type genes were amplified from local Bt isolates of Pakistan. The 2-kb full length *cry2Ac* and *cry2Ad* type genes were cloned, sequenced and deposited to EMBL with accession numbers *cry2Ac8* (AM421903), *cry2Ac9* (AM421904), *cry2Ac12* (AM689532), *cry2Ad3* (AM268418) and *cry2Ad4* (AM490199). It has now been named as Cry2Ac8, Cry2Ac9, Cry2Ac12, Cry2Ad3, Cry2Ad4 proteins by *B. thuringiensis* Delta-Endotoxin Nomenclature Committee. These *cry* genes were sub-cloned in pET22b expression vector. To assess the toxicity of Cry2Ac and Cry2Ad type proteins, BL21 (codon plus) strain of *E. coli* was further transformed with the recombinant plasmids. Proteins expression was analyzed on 12% SDS-PAGE. The partially purified Cry2Ac and Cry2Ad inclusion bodies, used for insecticidal activity against species of Lepidoptera (*Helicoverpa armigera*, *Spodoptera litura*) and Diptera (*Aedes aegypti*, *Culex quinquefasciatus*). Cry2Ad3 showed maximum mortality against *C. quinquefasciatus*, *A. aegypti* and *H. armigera* larvae. The weight loss among Cry2Ac8 treated *S. litura* insects were observed than other tested Cry toxins. The insolubility of the partially purified inclusion bodies plays an important role in mild toxicity of these Cry toxins.

DNA BARCODING OF INDIAN FLYING FOX (*PTEROPUS GIGANTEUS*), MITOCHONDRIAL *COI* BASED STUDY FROM AZAD JAMMU & KASHMIR, PAKISTAN

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DNA barcoding; the Cytochrome C Oxidase 1 gene (*COI*) is a genetic marker used as uniform, testimonial certification and reliable evidence for a universal species-level bio-cataloging system compared with the morphological identification. Current research emphasis on the DNA barcoding of *Pteropus giganteus* in Azad Jammu and Kashmir, Pakistan. These sequences were amplified and PCR products were sequenced and examined by bioinformatics softwares. Conspecific and congeneric, K2P nucleotide deviation and nucleotide composition, number of haplotypes and haplotype diversity was estimated. All of the five studied samples of *P. giganteus* were G-deficient (19.3 percent) than C (27.7 percent), A (25.3 percent) and T (27.6 percent). The calculated haplotype and nucleotide diversity were 0.033% and 0.51% respectively. The mean intraspecific K2P distance was 0.01% with high rate of transitional substitutions. Study revealed that *P. giganteus* (R=2.01) deviated from the neutral evolution (R=0.5). It was determined from these conclusions that *COI* gene is favorable marker for identification of bat species than nuclear genes due to its distinctive characteristics and may serve as landmark for the identification of interconnected species at molecular level and in determination of population genetics.

MOLECULAR CHARACTERIZATION OF *WALLAGO ATTU* OF AZAD JAMMU AND KASHMIR, PAKISTAN

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Fishes are the utmost varied and rich vertebrates and are scattered throughout the water in world because of their marvelous variation in ecological activities, bodily processes, structure and anatomic features. Due to biotic,

abiotic and Anthropological activities most of the species become endangered and extinct. Scientists are trying to develop the molecular techniques to conserve and manage the germplasm. Current research work has been focused on the molecular characterization of *Wallago attu* (Freshwater shark) in Azad Jammu and Kashmir by using molecular techniques. The fish samples were identified as *Wallago attu* on the basis of external morphology. Barcoding and phylogenetic analysis was conducted by using four conserved sequence of mitochondrial DNA of *Wallago attu* such as COI gene, *Cytochrome b* gene sequence, D-Loop or control region and 16 S rRNA gene sequence. These sequences were amplified and PCR products were sequenced and examined by bioinformatics software. Conspecific and congenric, K2P nucleotide divergence was estimated. Number of haplotypes, haplotype diversity and nucleotide diversity was also calculated. It is concluded that these genes in mtDNA is favorable markers for identification of fish species than nuclear genes due to its distinctive characteristics and may serve as landmark for the identification of interconnected species at molecular level and in determination of population genetics. These sequences including COI gene, *Cytochrome b* gene, D-Loop or control region and 16 S rRNA are the effective tools for identification of species as well as for determination of genetic diversity.

MOLECULAR CHARACTERIZATION OF *GLYPTOTHORAX KASHMIRIENSIS* OF AZAD JAMMU AND KASHMIR, PAKISTAN

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Fish is a diverse group of animals which are highly specific in terms of their aquatic habitat and cover approximately 50% of vertebrate species identified so far. Still, numerous fish species are unidentified and are becoming extinct due to increased anthropogenic activities. Scientists are trying to identify the species and conserve the threatened or endangered species. DNA barcoding is a method to establish such DNA sequences that can be used for identification, discrimination or taxonomic classification of organisms. The present study was aimed to identify the endemic fish of Jhelum River on the basis of both morphology and genetic analysis and to create the barcode sequences for gene bank. Fish samples were collected from Langarpura (Jhelum River, Muzaffarabad AJ&K) and identified by using combined techniques of Morphometry (Jayaram, 2010) and identified it as *Glyptothorax kashmiriensis*. For molecular identification, the DNA barcoding by mitochondrial COI gene and D-Loop gene is used. DNA was extracted through Phenol Chloroform-Isoamyl alcohol method and amplified through polymerase chain reaction using appropriate primers for Mitochondrial COI and D-Loop genes. Sequencing was done and total number of mutable sites in sequence alignments, Nucleotide diversity, haplotype formation number of polymorphic sites (S) and total number of mutations (η) were estimated using respective softwares. It is concluded from current research that COI is effective gene sequence and help in species identification.

STUDIES BASED ON MITOCHONDRIAL DNA BARCODE GENE *COI* OF THREE SPECIES OF SEA SNAKES (*HYDROPHIINAE*) FOUND IN COASTAL WATERS OF PAKISTAN

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Sea Snakes are unique and highly specialized organisms found in the Indian Ocean and hence found providentially in Pakistani waters. It is important to use DNA barcode and genetic markers such as the *COI* gene for the systematic and phylogenetic studies. During the current study, three species of sea snakes namely *Hydrophis schistosa*, *Hydrophis caeruleus* and *Hydrophis (Pelamis) platurus* were collected from coastal waters of Sonmiani Bay and Mubarak Village. Morphological and Molecular studies were conducted to identify and observe the abundance of biodiversity of sea snakes in Pakistani Waters. Morphologically, the three snakes varied in length, weight, color patterns and shape of heads in general. *H. schistosa* was found to be 2.8 ft. long with pale and green-gray ventral surface and whitish yellow dorsal surface. It was followed by *H. caeruleus* was found to be 3.5 ft.

long, with lighter body weight as compared to *H. schistosa* with a lean body which had visible black blotches on deep olive yellow base. *H. (Pelamis) platurus* was observed 1.8 ft in length with strikingly black ventral and bright yellow dorsal surface without any pattern. To authenticate the morphological findings, molecular studies were conducted through the use of mitochondrial *COI* gene. The identification and confirmation of all three species based on coding region of mt-DNA Cytochrome Oxidase I (*COI*) gene. The sequence similarity (more than 95% similarity) to the gene sequence of three species determined by using the online NCBI Blast tool. The procured sequences submitted to Gene bank for data record.

GENETIC CHARACTERIZATION OF MITOCHONDRIAL DNA CONTROL REGION OF WAKHI POPULATION OF PAKISTAN

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The mtDNA control region assists to find out the immigration trail of a population throughout the history along with other related uni parental genetic constitution. Analysis of mtDNA haplogroups has been proven valuable in determining not only the evolutionary account of human populations but also the turns the trick in favor of crime investigators to identify a human profile where conventional STR typing comes with limitation to find a hit. This research gives insight of mitochondrial DNA haplotypes data of CR (control region) covering the area from 16024bp to 576bp of mitochondrial DNA of Wakhi population of Hunza valley from Pakistan. Samples of 40 unrelated Wakhi from upper Hunza were sequenced and their sequences encapsulating mtDNA control region was compared to rCRS (revised Cambridge reference sequence) to check maternally inherited DNA variation at genetic level in this population. The results showed 39 haplotypes were unique and only 1 haplotype was shared in all 40. The haplotypes corresponded to 67.9% West Eurasian haplogroups followed by the Middle East and variety of Asian haplogroups exhibiting admixed maternal genetics of this population. Wakhi population comes with high genetic diversity (0.998) in turn lowest random match probability (0.026) and random match probability (0.974). This study gives interesting insights about genetics of Wakhi population and is also a contribution to mtDNA control region data of Pakistani populations for applications in forensic science.

FUTURISTIC APPROACH OF STEM CELLS APPLICATIONS IN CLINICAL RESEARCH

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In human body various tissues categories bring into exist, by the non distinct cells which when develop into the distinct cells are named as stem cells. The renewability potential in the mitotic cell division process to rebuilt themselves as well as differentiation potential, to produce distinct cells variety which are responsible for classification of these cells into different groups. Stem cells play unique role, for muscles, bones, brain, nerves, skin, blood, or other organs development, growth, prolongation couple with rehabilitation. The ever so extensively used stem-cell therapy is bone marrow transplantation, to a limited extend therapies from umbilical cord blood derivatives are practical. For stem cells different sources development, and to apply stem-cell treatments for neurodegenerative diseases together with some other disease like diabetes or cardiac, research is in the way of progress. The foremost aim of using stem cell technology is to put " a patient in a dish". Medical together with dental practice will be advanced through the technical use of stem cells as its impact is vast. As a clinical standard stem cells are playing a unique role, as for leukemia hepatopoietic stem cells are transplanted, for corneal disorders as well as for burns epithelials, epithelial stem cells based treatments are applied. Additionally stem cell based therapy also play unique role in disease modelling and in last degree ADM and for cancer cure cancer stem cells are vital, for instance liver cancer stem cells can be radically treated liver cancer. Although LCSCs recognition can be done through LCSC markers analysis and stem cells are in the way of progress to mimic genetics together with metabolic complexities of human beings. Here, we review the current status and future perspective of utilizing hPSCs, specifically induced. pluripotent stem cells (iPSCs), in basic and clinical AMD research, but also inevaluating potential treatment options.

We provide an outline of concepts for disease modelling and summarize ongoing and projected clinical trials for stem cell-based therapy in late-stage.

MOLECULAR CHARACTERIZATION OF *CRY4A* GENE FROM *BACILLUS THURINGIENSIS*

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The mtDNA control region assists to find out the immigration trail of a population throughout the history along with other related uni parental genetic constitution. Analysis of mtDNA haplogroups has been proven valuable in determining not only the evolutionary account of human populations but also the turns the trick in favor of crime investigators to identify a human profile where conventional STR typing comes with limitation to find a hit. This research gives insight of mitochondrial DNA haplotypes data of CR (control region) covering the area from 16024bp to 576bp of mitochondrial DNA of Wakhi population of Hunza valley from Pakistan. Samples of 40 unrelated Wakhi from upper Hunza were sequenced and their sequences encapsulating mtDNA control region was compared to rCRS (revised Cambridge reference sequence) to check maternally inherited DNA variation at genetic level in this population. The results showed 39 haplotypes were unique and only 1 haplotype was shared in all 40. The haplotypes corresponded to 67.9% West Eurasian haplogroups followed by the Middle East and variety of Asian haplogroups exhibiting admixed maternal genetics of this population. Wakhi population comes with high genetic diversity (0.998) in turn lowest random match probability (0.026) and random match probability (0.974). This study gives interesting insights about genetics of Wakhi population and is also a contribution to mtDNA control region data of Pakistani populations for applications in forensic science.

6. PHYSIOLOGY

ROLE OF VITAMIN D₃ AND FORMIC ACID SUPPLEMENTATION IN IMPROVING THE GROWTH PERFORMANCE AND DIGESTIVE ENZYME ACTIVITIES IN *LABEO ROHITA* FINGERLINGS

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The present research was conducted to investigate the effects of formic acid and vitamin D₃ on the growth, muscle proximate composition, activities of intestinal digestive enzymes and bone mineralization in the *Labeo rohita* fingerlings. Four test diets i.e. T1, T2, T3 and T4 containing formic acid (%) and vitamin D₃ (IU/Kg) at the level of 0,0; 2,0; 0,5000 and 2,5000, respectively, were prepared. Fish was fed twice a day for 3 months. At the termination of the feeding trial, 8 fish were harvested from each experimental unit and samples of muscle, intestine and bones were taken. The supplementation of vitamin D₃ enhanced the growth in terms of final weight, absolute weight gain, weight gain% and specific growth rate, same results were obtained in formic acid fed fish. The dietary vitamin D₃ formic acid supplementation enhanced the bone mineral contents including P, Ca, Mg, Na, K, Zn, Cu, Fe and Mn. The dietary vitamin D₃ enhanced the intestinal digestive enzyme activities as well. In conclusion, vitamin D₃ and formic acid supplementation improved the growth performance, muscle proximate composition, bone mineralization and intestinal digestive enzymes activities in *L. rohita* Fingerlings.

HISTOPATHOLOGY OF UMBILICAL CORD: ROLE OF ANGIOTENSIN-I CONVERTING ENZYME (ACE) POLYMORPHISM IN SUSCEPTIBILITY TO PREECLAMPSIA

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Preeclampsia (PE) is a pregnancy-related disorder involving multiple organ systems, characterized by increased hypertension, and proteinuria after 20 weeks of gestation. It is a multi-factorial and multi-systemic, disorder complicating 5-7% pregnancies and one of the leading causes of fetal/maternal morbidity and mortality worldwide. PE is associated morphologically, biochemically and functionally with abnormalities in the umbilical cord. Among several pathophysiological mechanisms, component of Renin-angiotensin aldosterone system (RAAS) i.e. Angiotensin-converting enzyme (ACE) which converts angiotensin I (inactive) to angiotensin II (active) has been implicated in the development of PE. The aim of this study is to evaluate the risk of I/D polymorphism of the ACE gene and morphometric/ histopathological degenerations induced in the umbilical cord (UC) of women with preeclampsia (PE) when compared with healthy controls. For this purpose, blood samples (PE=66, controls=54) and tissue samples of the umbilical cord (PE=30, controls=30) were collected and several genotypic, histological and anthropometric parameters were measured. Genotyping for ACE I/D polymorphism was performed by polymerase chain reaction (PCR) based gel electrophoresis. While, histological cross-sections of UC were photographed and measured by recording following variables: distance of outer layer from Wharton's jelly (in both arteries and vein), outer and inner layer thickness of arteries, the

layer thickness of vein and lumen area (of both vein and artery). Data was statistically analyzed through calculating mean \pm SEM, chi-square analysis, unpaired student t-test and odds ratio. The mean body mass index (BMI), maternal age, blood pressure, age at first marriage and age at first childbirth of PE group revealed significant increase when compared with the control while the significant decline in PE group was observed in case of gestational age. Also, the higher percentage of consanguineous marriages, family history of PE and pregnant with twins was observed in PE group. Histological study revealed significant increase ($p < 0.001$) in distance from Wharton jelly (in both artery and vein) and outer layer thickness of vein; significant reduction ($p < 0.01$ and $p < 0.05$) in the lumen area of artery and vein and non-significant change was observed in case of arterial outer and inner layer thickness. The frequencies of DD, ID and II genotypes among PE patients were 63.63%, 21.21% and 15.15% versus 51.85%, 42.59% and 5.55% for the control group, respectively which were statistically significant. The genotypic frequency showed a significant difference ($p < 0.02$) while allelic frequency showed the non-significant difference between both groups. The I/D polymorphism of the ACE gene seems to influence the therapeutic outcome in PE women. The histomorphometric changes/abnormalities observed in the umbilical cord of PE group can be a good predictor of intrauterine growth retardation and its reflection on the outcome of neonates. Further studies are required to elucidate the functional roles of ACE gene in a larger population.

EXPOSURE OF BISPHENOL A AND ITS ALTERNATIVES BPB, BPF AND BPS IMPAIRS SUBSEQUENT REPRODUCTIVE POTENTIAL IN ADULT FEMALE SPRAUGE DAWLEY RATS

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Bisphenol A (BPA) and its analogues (BPB, BPF and BPS) are widely used in household and industrial products. All analogues have higher affinity for estrogen receptors and mimic its actions. The current study aims to investigate the comparative effect of bisphenol A analogues (BPB, BPF and BPS) on reproductive health in adult female rats. Eighty-five post weaning female rats (90 \pm 15) were divided into seventeen groups and assigned for different treatments. 1 ml of saline was administered intraperitoneally to control. Treated groups were injected with different concentrations (0, 0.05, 0.5, 5 and 50 mg/kg) of bisphenol A, B, F and S for 28 days. On day 29, rats were dissected and blood and ovarian tissues were collected. Body weight as well as weight of reproductive and body organs was noted. Blood plasma was separated, later used for hormonal analysis while ovaries were used for histology and biochemical analysis. Levels of plasma testosterone, estradiol and progesterone were analyzed through enzyme linked immunoassay. The present studies showed adverse morphological and histopathological alterations in rat ovaries. BPA and all its alternatives showed non-significant increase in body weight among all maximum dose treated groups (50mg/kg). While results of weight of reproductive organs, Gonadosomatic index showed significant reduction in both 5 and 50 mg/kg bisphenol A and its analogues when compared with control and other groups. A remarkable decrease was observed in pre-antral, antral, pre-ovulatory and corpus luteum count while rise in atretic and cystic follicles was seen in the ovaries of BPA and its alternatives (5 and 50 mg/kg). Catalase (CAT), super oxidase (SOD) and peroxidase (POD) levels in ovarian tissue were found significantly lowered ($P < 0.05$, $P < 0.01$ and $P < 0.001$) in all treatment groups (0.05, 0.5, 5 and 50 mg/kg) when compared with control and among themselves. Similarly, very prominent rise in thiobarbituric acid reactive substances (T-BARS) and reactive oxygen species (ROS) showed significant increase in groups exposed with (0.05 mg/kg) of bisphenols when compared among themselves. While, (0.5, 5 and 50 mg/kg) of all bisphenols resulted in significant increase ($P < 0.05$, $P < 0.01$ and $P < 0.001$) when compared with both control and among themselves. Plasma testosterone and estradiol levels were significantly increased, while plasma progesterone concentrations were significantly reduced in the highest dose treated groups than control. The current data suggests that BPA and its analogues exposure during pre-pubertal stage have the potential to induce oxidative stress as well as induce histo-morphological alterations in follicular development.

THE EFFECT OF OBESITY ON INFERTILITY RELATED HORMONES AMONGST YOUNG FEMALE IN HYDERABAD AND ADJOINT AREAS

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Obesity is a disorder and is an important health issue mostly occurs amongst the women of reproductive age. Obesity and overweight involve abnormal and negative effects on health if the Body Mass Index (BMI) is equal to or greater than 25kg/m², considered overweight, whereas equal to or greater than 30kg/m² considered as obesity. The Infertility is the failure of a couple to conceive a pregnancy after trying to do so for at least one full year with unprotected intercourse and without using any other method of contraception. This problem worldwide carried a value of 10-15% in every year. It was reported to be present in 21.9% of Pakistani population in 2003. Some factors that are influenced such as Ovulatory defects and unexplained cause account for >50% of infertile etiologies and overweight and obese women has topped in some developed countries more than 50 % also. In the present study data sampling was collected from LUMHS hospital OPD and ISRA hospital we collected 5ml fasting venous blood sample from 60 Obese Females on day 2 or day 3 of menstrual cycle. The distribution of frequency in obese and overweight 60 infertile females were 67% obese and 33% were overweight according to BMI scale. The medically and biochemically analysis of data of overall 60 cases of infertile females were examined by BMI scale, Glucose, Blood pressure (systolic and diastolic), having to mean and standard deviation were 32.96 ± 4.4, 95.0 ± 2 0.0, 120 ± 10.0 and 77.6 ± 10.18. The present research suggested that the obesity is associated and responsible for causing infertility in female according to BMI scale, blood sugar and Blood pressure reports along with causing factors for hormonal changes in young female. So the further hormones associated with infertility will be analyzed and will present in final seminar.

FERTILITY OF CRYOPRESERVED BUFFALO SEMEN CAN BE IMPROVED BY SUPPLEMENTATION OF ARACHIDIC ACID IN EXTENDER

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The aim of this study was to determine the *in vivo* fertility of cryopreserved Nili-Ravi buffalo semen by supplementation of arachidic acid in extender. For this purpose, semen was collected from three adult Nili-Ravi buffalo bulls (*Bubalus bubalis*) of similar age group with artificial vagina (42°C) for five weeks (replicates; n=30). Semen was diluted in *tris*-citric acid extender containing 0.0 (control), 20.0, 25.0 and 30.0 ng/mL of arachidic acid at 37°C (>1 mL volume, >60% motility, >0.5 billion/mL concentration) and cryopreserved using standard procedures. Percent sperm progressive motility, plasma membrane integrity, liveability and viability were higher (P<0.05) in extender containing 20.0 ng/mL of arachidic acid compared to 25.0 ng/mL, 30.0 ng/mL and control. However, sperm chromatin integrity was equally improved in experimental extenders containing arachidic acid compared to control. Sperm abnormalities were reduced in experimental extender containing 20 ng/mL of arachidic acid compared to other experimental extenders containing arachidic acid and control. In experiment 2, a total of 533 inseminations were performed with the extender containing best level of arachidic acid (20 ng/mL of extender). *In vivo* fertility was significantly improved in buffaloes inseminated with semen containing 20.0 ng/mL of arachidic acid (58.64%) compared to control (46.06%). In conclusion, supplementation of arachidic acid at 20.0 ng/mL in extender significantly improved the post thaw quality and *in vivo* fertility of cryopreserved Nili-Ravi buffalo bull spermatozoa.

HISTOPATHOLOGY OF PLACENTA AND ASSOCIATION OF VARIOUS RISK FACTORS TO PATHOPHYSIOLOGY OF PREECLAMPSIA IN PAKISTAN

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Preeclampsia (PE) is hypertensive pregnancy disorder which is one of the leading causes of maternal and neonatal morbidity and mortality. Present study was designed to identify various risk factors for PE in pregnant women. Total 400 blood (PE/controls=200) and 90 placental tissue samples (PE=50, controls=40) were recruited. History, blood and tissue samples were collected from each subject with informed consent. Data was analyzed, and laboratory tests were done for blood. Results were statistically analyzed by Independent sample t test, Chi square test and the odds ratio. Significant risk factors for PE were Age (p 0.002), BMI (p=0.047), Gestational age (p <0.001), blood pressure (p <0.001) and weight of child (p <0.001) Headache (OR: 3.63), swelling in hands and face (OR: 5.53), excessive weight gain (OR: 4.47), history of preeclampsia in previous pregnancy and in family (OR: 13.87; OR: 6.69). Blood markers including total leucocyte count (TLC) (p=0.028), Haematocrit (p=0.016), alkaline phosphatase (p<0.001), serum urea(p<0.001) and uric acid (p<0.001) total bilirubin (p=0.019) and AST (p=0.012) and urine proteins (p<0.0001) were significantly different in both groups. Abnormal villi, more syncytial knots (SK) and significant decrease in elongated and large villi in PE placentas as compared to normal placentas were observed. Other parameters including area, perimeter, circularity, ferret and minimum ferret diameter were reduced in elongated and large VP of PE placentas as compared to control group. In conclusion, clinical and histological risk factors should be valued in early diagnosis and prevention of PE. Moreover, such risk factors should be considered in the design of future studies dealing with timely management of PE in Pakistan.

STUDY OF REPRODUCTIVE TOXICITY INDUCED BY ENDOCRINE DISRUPTORS IN ADULT ZEBRA FISH

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Endosulfan and Imidacloprid are commonly used neonicotinoid pesticides in agriculture. Their presence in any environment may lead to severe damage to ecological systems, especially in aquatic habitat. The current study was designed to explore the toxic effects of imidacloprid and endosulfan in terms of histological alterations in gonads and DNA damage in male and female zebrafish. Adult zebrafish were divided into four groups; Control group (C), Group 1 (G1), Group 2 (G2) and Group 3 (G3). Each group was exposed to different concentration of endosulfan (0.1, 0.5, 1µg/L) along with imidacloprid (1mg/L) for 21 days. Gonadal tissues were sampled at 7, 14 and 21 days of exposure. Zebrafish gonads exposed to combination of pesticides were normal in external appearance and showed increased body weight and body length. Histopathological and morphometric analysis manifested that control group showed normal morphology of primary, cortical alveoli, vitellogenic and mature follicles in ovaries of zebrafish. On day 7, zebrafish exposed to endosulfan and imidacloprid showed increased follicular diameter with an increase in concentration of endosulfan while diameter of atretic follicles were decreased. On day 14 and 21, diameter of all type of follicles were increased as compare to control in a dose dependent manner. Cortical alveoli follicles showed significant reduction in diameter at 21st day. Histopathological alterations observed in ovary of treated fish after 21 days of exposure include increased membrane folding and more disrupted follicles. The number of primary follicles were decreased with an increase in endosulfan concentration. Percentage of vitellogenic and mature follicles were reduced after 7 days while they were significantly increased after 21 days of exposure. Number of cortical alveoli and atretic follicles were significantly increased. Stereological analysis of zebrafish testes showed all type of germ cells in control including spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids and spermatozoa. The number of spermatogonia, primary spermatocytes and secondary spermatocytes were significantly increased with an increase in endosulfan exposure. Number of spermatid and spermatozoa were reduced in a dose dependent manner on 7, 14 and 21 days. This study showed that different types of germ cells get affected in various manner at different exposure. Comet assay was performed to investigate the extent of DNA damage in adult zebrafish. Time and dose dependent DNA damage was observed with increasing concentration of endosulfan (1µg/L) in gonads of

both male and female zebrafish. Based on our results, we can conclude that endosulfan and imidacloprid cause toxicity in male and female zebrafish and they play important role in disrupting endocrine functions in adult zebrafish.

STUDIES ON THE OOCYTE DEVELOPMENT IN THE FISH (*OREOCHROMIS NILOTICUS*) NILE TILAPIA, JAMSHORO, SINDH

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Oocyte development is a very important biological phenomenon to generate the eggs. In this context, an attempt was made to study the pattern of oocyte development in this fish *Oreochromis niloticus* which is a group spawner fish and spawned many times in a particular breeding period. The fish were collected on different days during April-May 2018. This fish on every catch was sacrificed and ovarian tissue was saved for histological examination. As per histological results, the ovaries of the fish caught on April 12 were contained of either gravid follicles or few eggs in which the ovulation had already occurred were also available. It was also noted that the oocytes of previtellogenesis state were also available in the vicinity of the large follicle. The condition of the ovary of the fish collected after one week in April 19 shared maximum stage **iv** and **v** follicles along with few atretic follicles. More or less same condition was noted in the fish caught on April 30 and May 5. It is concluded from the studies under taken that the fish *Oreochromis niloticus* breeds continuously in gravips throughout the breeding season. One batch of the eggs when reaches final maturations, gets out followed by another group of oocytes get matured in another week and released the eggs according. This pattern will be continued till the environmental conditions required to boost the reproductive cycle are favorable.

RELATIONSHIP OF SERUM LEPTIN LEVELS WITH WEIGHT GAIN AFTER MENOPAUSE

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The prevalence of overweight or obesity in middle-aged women, around and after menopause is high and is rising worldwide, more rapidly in women over the age of 40 years. This study was planned to examine the differences in BMI and serum leptin concentration of obese and control (normal weight) post-menopausal women. This experiment was carried out in Karachi (January-April 2015) and employed the cross sectional design. We included women aged 45-60 years with ceased menstruation for at least 12 month and had a natural menopause. A total of 84 postmenopausal women were volunteered for the study which included control (n=13) and obese (n=71). The home visits included the use of a face-to-face questionnaire and written consent was obtained from each subject. Weight and height was taken then BMI was calculated. Leptin hormone was analyzed by Human ELISA kit. Statistical procedure involved unpaired t-test and P values below 0.5 were accepted significant. Our findings evaluated the significant differences in the average BMI values of control (22.14 ± 0.023) kg/m² and obese postmenopausal women (35.93 ± 0.72) kg/m². BMI of obese females were significantly higher than control females ($P < 0.001$). Similarly, the serum leptin levels of obese women (55.43 ± 2.14) were significantly higher than control women (22.55 ± 4.53 ; $P < 0.001$). The hormonal changes across the menopause substantially contribute to increased abdominal obesity. Serum leptin concentration was positively and significantly related with BMI.

PREVALENCE AND SOCIO-ECONOMIC DETERMINANTS OF INFERTILITY IN FEMALES AT HYDERABAD DISTRICT OF SINDH PAKISTAN

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The purpose of the study was to investigate the actual reasons of infertility in females. Infertility is a world health issue which affecting approximately 9-12% of couples. It is multifactor problem with social economic and cultural consequences. The study was consisted of 90 patients infertile females who sought medical help in private and government hospitals of Hyderabad for the period of 2 months. Collections of data was performed by means of a specifically designed questionnaire. The sample studied consisted of 90 patients infertile females who were suffered of infertility due to these reasons hypertension 10%, pcOS 25%, blockage of fallopian tubes 8%, diabetes 10%, malnutrition's 5%, depression 20%, smoking 2%, and 20% of females are not fertile because of their age. Out of 90 patients only 40 % are graduate and other are less education status. The main causes of female infertility is depression, pcOS and age.

CO-RELATION OF HYPERTENSION AND DIABETES IN POPULATION OF PESHAWAR, PAKISTAN

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Hypertension and diabetes are the common diseases which occur together in the general population and are refer to as comorbidities. The hypertension in diabetic population is twice more than in non-diabetics. Both the diseases have same risk factors which determine the levels of hypertension. In this study, levels of hypertension in diabetic male and female population of Peshawar was detected. Further, correlation of hypertension with diabetes and socio-economic condition was also explored. Data was collected from 151 patients of Hayatabad Medical Complex, Peshawar. This study revealed that, hypertension in diabetic patients was 82% (78 % in males and 85.5% in females). Several risk factors like obesity was found to be 29%, smoking 44% (40 % in males and 4% in females). 93% patients were detected with high level of stress. Hypertension and diabetes both lead to kidney disease and present study also found 70% kidney diseases among the patients. Most of the hypertensive diabetic patients belongs to the middle class of socio-economic condition. Hypertension is growing more and more common along with diabetes and female are more susceptible to high blood pressure levels than man in older age. High level of stress has been found among both the groups of males and females that belongs to the middle class of socio-economic condition.

STUDY OF BIOCHEMICAL PARAMETERS IN DIABETES MELLITUS PATIENTS FROM SOUTHERN PUNJAB, PAKISTAN

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Diabetes Mellitus (DM) is a major health problem all over the world. Electrolyte imbalances (EI) are associated with hyperglycaemia in diabetic patients. Every diabetic patient needs proper evaluation of electrolyte imbalance for rational prevention and management of disease. The aim of this study was to determine the common types of electrolyte imbalances in patients of diabetes admitted in the department of Medicine, Nishter Hospital Multan. A total of 250 subjects including 100 controls (50 males and 50 females) and 150 admitted patients of diabetes (75 males and 75 females) with average age 62.4 ± 0.69 ranged 40-85 years were interviewed, physically examined and Blood Samples were collected for Fasting Blood Glucose (FBG) level and biochemical analysis of electrolytes including Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-). Fasting Blood Glucose (FBG) was significantly high in diabetic patients compared to controls. Serum sodium and chloride levels were significantly low while K^+ level was

significantly high in patients. Correlation analysis of FBG showed positive association with k^+ in diabetic females. Analysis of these biomarkers is important for timely management of diabetes due to electrolyte imbalance that is major cause of morbidity and mortality.

PREVALENCE OF HYPERTENSION IN TEHSIL KHWAZA KHELA, BABUZAI AND MATTA, DISTRICT SWAT

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Hypertension is common cardiovascular risk factor worldwide and highly prevalent among the adults (40%), with an age above 40 years. The prevalence of hypertension was determined among the people of three tehsils of District Swat. Total of 1000 people were examined both hypertensive and normal from Tehsil Khwaza Khela, Babuzai and Matta for the determination of incidence ratio of hypertension. It was correlated with their risk factors such as age, gender, economic status, qualification, exercise, sleeping habits and food taking. Standard questionnaires were filled from subjects of age ranges from 15-94 years (mean: 54.5) including 713 males and 287 females. The high rate of prevalence of hypertension was found in age groups above 45 years (85%) where, as lower in age groups below 34 years (9.6%), on gender based its ratio was 57% in females and 30% in males, found a significant difference between age and gender groups. On qualification based the high prevalence was found in illiterates (57%) and lower was found in graduates (10%), a big difference was also received in economic level as 81% in upper class, 70% in lower while 34% in middle class. The ratio of hypertension was about 21% below in subjects who doing physical exercises and 29% above in subjects having stomach problems but the sleeping cycle not significantly affect hypertension. The most prominent symptoms found in hypertensive subjects was fatigue and headache (92%), confusion and anxiety (88%), sweating (81%), dizziness (75%), irregular heart beat (72%) and a lot of other related symptoms. The prevalence of hypertension was high comparatively in study site. But the people need awareness to avoid hypertension.

PREVALENCE AND RISK FACTORS OF TYPHOID FEVER IN PESHAWAR, KHYBER PAKHTUNKHWA, PAKISTAN

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Typhoid fever, caused by *Salmonella typhi* is an important and persistent health problem in poor and developing countries like Pakistan which leads to high mortality and morbidity rates. It is characterized with sustained fever and rose spots and is caused by ingestion of contaminated food and water which is enhanced by poor sanitation. Three hundred and fifty patients were included in this study. Demographic data were collected from 300 individuals from different hospitals and clinical laboratories and 3ml blood was collected from 50 suspected patients having sign symptoms of typhoid. Serum was separated and was carried out to Molecular Biology and Virology laboratory, Department of Zoology, university of Peshawar for diagnosis of typhoid fever with widal and typhidot tests using the method of manufacturers. Analysis showed that females (56%) are highly exposed to typhoid. In addition illiterate, poor and children are most vulnerable to the disease. They are mostly treated with typhidot test and were positive with IgM (74.6%) and IgG (7.9%) stages. And the rest were positive with both IgM and IgG stages. Typhoid occurrence and risk prediction in our study has the potential to advise public health professionals and researchers to identify risk factors associated with typhoid and take participation in control of many diseases like typhoid.

INCIDENCE AND RISK FACTORS OF PNEUMONIA IN VULNERABLE CHILDREN OF MUZAFFARABAD

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Globally pneumonia records for almost one in five demises among children below 5 years. About 450 million individuals affected by pneumonia per year and it is a common disease occurring throughout the world. Pneumonia is the main cause of death amongst all age peoples causing 4 million casualties (7 % of the world's total death) annually and its rate is highest in children. The highest figure of kid's pneumonia incidents happens in the Asia-Pacific region. In Pakistan, due to long term winter duration people (children) at high altitude mostly suffered from pneumonia. The current study was aimed to find the incidence and risk factors of pneumonia in vulnerable children of Muzaffarabad Azad Jammu and Kashmir, Pakistan. Blood sampling of suspected Pneumonia cases was done. Hematology analyzer was used for complete blood analysis. Infected children were further processed by chest X-ray. Among all 326 children, 200 (61 %) children were infected with pneumonia and the prevalence rate is highest in male children (60.5 %) while in female children it was 39.5%. Illiteracy, low socioeconomic status, malnutrition, indoor air pollution, mud houses and high altitude have been found major contributors of pneumonia in children. Such a high prevalence rate is alarming health concern in children of Muzaffarabad Division. This high rate is due to the lack of awareness about this infectious disease and its risk factors. To control this, parent's education and doctor's method of treatment is of important concern.

COMPARITIVE EFFECT OF OVAPRIM AND CARP PITUITARY GLAND EXTRACT ON BREEDING CAPABILITY OF RAHOU AND MORI

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The Current studies compensate usefulness on the Carp (PGE) and the artificial hormone such as Ova prim on induce spawning, achievement and time of induced breeding on Rohu and Mori. The dose of 2 Mg/Kg of the body weight of (PGE) hormones to males and 4 Mg/kg of body weight of female were used. Ova prim were introduced with 0.5 ml/kg of the body weight of females and 0.25 ml/kg of the body weight for males. The induce spawning success was more in Ova prim induced fish by means of better presentation as compared to the PGE-induced individual noted at every stage of spawning as well as latency phase, ovulations, fertilizations, hatchings and incubations stage. The latency time take place within 10 hour in the Ova prim induced individuals, while, the latency time up to 15 hours in PG given individuals. Correspondingly, ovulations rates were 90 % for Ova prim given fish and lesser 78.70% for (PGE) of the given individuals. Ova prim given fishes greater proportion of fertilization was experimentally observed in 86.70% is compare 69.20% in (PGE) induce individual. In contrast hatching rate was 76.9 % in eggs spawned from ova-prim induce individual as compare to 72.7% in (PGE) given fishes. In conclusion this result show that the Ova prim treated fish gives better result in contrast to the (PGE) dose given fish in conditions eggs production, fertilizations and hatchings rate upon the (*Labeo rohita*) And (*Cirrhinus marigala*).

EXAMINE THE EFFECTS OF ALLOXAN ON LIVER, BODY WEIGHT AND HEMATOLOGICAL PARAMETERS OF MICE

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Diabetes mellitus is a metabolic disorder caused by dysfunction of pancreatic β cells. Different types of herbal

medicines are used to prevent diabetes for a long time. Therefore, the present study was designed to evaluate the hypoglycemic effect of herbal extract (combination of Cinnamon and Fenugreek 2:1) 200 mg/kg body weight and to compare it with the hypoglycemic effect of an allopathic drug Metformin 650mg/kg body weight. Procyanidin and 4-hydroxyisoleucin are bioactive component of Cinnamon and Fenugreek which shoes antidiabetic activity. Mice were divided into 4 groups. First group was –ve control group, second group was +ve control group and third was treated with herbal extract and forth group was treated with Metformin. Single intraperitoneally injection of alloxan monohydrate 200mg/kg body weight was given to Swiss Albino mice to induce diabetes (+ve control). Herbal Extract at the dose of 200 mg/kg was given to group III (Treated group) for 21 days. Metformin also was given to treated group IV at the dose of 650 mg/kg for 21 days. The bilirubin level was increased in +ve control group and after giving the herbal extract and metformin to treated group III and IV this level was significantly ($p < 0.05$) reduced progressively. Similarly, due to liver damage Serum Glutamic Pyruvic transaminase (SGPT), Serum Oxaloacetic (SGOT) and Alkaline Phosphate (ALP) was significantly ($p < 0.05$) increased in alloxan induced diabetic mice. After administration of extract and metformin their level was significantly ($p < 0.05$) decreased close to –ve control group. This reduction showed the effectiveness of herbal extract and metformin. But the metformin was more effective than herbal extract. In diabetic group liver section showed a high degree of damage. After treatment the normal architecture of liver section was observed. From the experimental findings it is concluded that the herbal extract exhibit hypoglycemic property although its effect is lower than that of allopathic drug metformin.

CORRELATION BETWEEN TESTICULAR MEASUREMENTS WITH AGE, BODY WEIGHT AND BODY SIZE OF KAMORI GOAT BUCKS

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The study was conducted on twenty apparently healthy Kamori goat bucks 9 months of age at Kamori goat farm Khudabad, Dadu. Investigations/sampling of bucks commenced at the age of 9 months and terminated 9 months after when they was 18 months of age to assess the relationship of body measurements with testicular measurements. The body weight of the experimental animals was measured in kilograms by the procedure as described by Akpa *et al.* (1998). Weighing was done at the beginning of the study and subsequently on monthly basis. Measurement of linear conformation traits were taken in centimeters using flexible tape as described by Alphonsus *et al.* (2009) and Boisot *et al.* (2002). Similarly as in body weight the measurement of linear conformation traits and testicular measurements were taken at the onset and subsequently on monthly basis. The simple Correlation between Testicular Measurements with Age and Body measurements were worked out (Becker, 1985). Maximum body weight (39.17 ± 2.00 kg), heart girth (116.43 ± 2.17 cm), stature (134.29 ± 3.18 cm), chest width (14.74 ± 0.63 cm), wither height (123.61 ± 2.62 cm), body depth (136.27 ± 2.95 cm), body length (102.53 ± 2.58 cm), rump width (26.93 ± 0.39 cm), testicular length (14.32 ± 0.75 cm), testicular circumference (29.86 ± 1.84 cm), testicular width (14.93 ± 0.92 cm) and testicular weight (125.23 ± 14.56 g) was observed for 18 months of age. Minimum body weight (20.75 ± 1.05 kg), heart girth (61.60 ± 1.15 cm), stature (71.05 ± 1.68 cm), chest width (7.80 ± 0.33 cm), wither height (65.40 ± 1.19 cm), body depth (72.10 ± 1.56 cm), body length (54.25 ± 1.36 cm), rump width (14.25 ± 0.20 cm), testicular length (7.57 ± 0.39 cm), testicular circumference (15.80 ± 0.97 cm), testicular width (7.90 ± 0.48 cm) and testicular weight (34.66 ± 4.04 g) was noted for 9 months of age. There was positive relationship of age with all body and testicular measurements of goat. This shows that all the studied parameters increased in a linear trend with growing age.

STUDY OF DIURNAL RHYTHM IN LEVELS OF LUTEINIZING HORMONE IN CATLA CATLA

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Light pollution disrupts the world's ecosystem. The rhythm of life is orchestrated by the natural diurnal patterns of light and dark, so disruption to these patterns impacts the ecological dynamics. Changes in the duration of photoperiod have been proved to be the primary and regular variable that may impel the driving function in determining the sexual periodicity in most fish species. The daily pattern of melatonin secretion is conserved among vertebrates, as it is low during the daytime and high during the night, and thereby serves as the internal neuro-hormonal signal of darkness which may be interrupted by continuous light exposure. Inhibition of melatonin rhythms can implicate suppression in gonadotropins (Follicle stimulating hormone and Luteinizing hormone). Present study was designed to investigate the effects of light pollution on diurnal profile of Luteinizing hormone (LH) in major carp catla (*Catla catla*) over the period of 16 hours at different points. A significant variability ($P < 0.05$) has been observed between values of LH at different sample points. Concentration of LH was found to be within the range of 0.0065 ± 0.0000 ng/ml to 0.03315 ± 0.00015 ng/ml. However, diurnal profile of LH was observed to be disrupted by continuous exposure of light. No specific pattern in levels of LH was observed in relation to continuously suppressed levels of melatonin. It can be inferred that light pollution disrupts the diurnal rhythm of LH in *Catla catla*. Therefore continuous exposure to light pollution in laboratory and farm conditions might interfere with research results. Preventive measures should be taken to avoid this interference.

SUCCESS RATIO OF ARTIFICIAL INSEMINATION IN COWS AND BUFFALOS IN TEHSIL MANSEHRA; A STEP TOWARDS CONSERVATION OF BETTER MILK BREED

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In Tehsil Mansehra farmers prefer to have cows because cows can survive cold temperature easily as compared to buffaloes which are adaptable to warm climate. Local breed of buffalos is adoptable to cold temperatures of Tehsil Mansehra. There is need to conserve either semen or crossbreed local breed to some better milk breeds of diverse origin so milk production of local specie can be increased to get higher market value than local breed. For increasing milk yield, crossbreeding through artificial insemination using semen of exotic breeds is beneficial. Dairy sector is an important sector for poverty alleviation and rural development in Pakistan. Dairy cows and buffaloes reproductive performance determines herd profitability, to get one calf per animal per year it is necessary to have cows and buffaloes impregnated each year. This places an increased pressure on the need to understand factors that are responsible for infertility, including both herd level management factors and individual cow and buffaloes health factors. Artificial insemination (AI) is an important technique, contributing to the improvement of livestock genetic potential through rapid dissemination of superior germplasm and faster genetic gain. The experiment was conducted at District Artificial Insemination Center, Mansehra for a period of January 2016 to June 2017 with 200 cows and 200 buffalos of different genotypes. Questionnaire was filled up to collect related data during artificial insemination (AI). All the data related to farm conditions was collected from the farmers and was analyze. The various factors investigated were: Effect of farm conditions on conception rate (feeding system, housing system, season, size of herd); Effect of animal type and quality on conception rate (species, cow breed, buffalo breed, milk production, age, body condition score, Lactation stage); Effect of estrus signs on conception rate (heat signs, type of mucus, swelling

of vulva, uterine tone); Effect of semen on conception rate (breed of bull/ox, type of semen, semen thawing, source of semen, site of semen deposit, passage of gun); Effect of A.I technician on conception rate (level of education of A.I technician. Subsequently pregnancy diagnosis was done through Progesterone test. The overall average conception rate observed in Tehsil Mansehra (68%) was higher than the average (48.06%) reported by Woldu et al. (2011) but lower than the report of researcher Berglund et al. (2006) who reported higher conception rate of 71%. Difference between studies can be attributed to multiple factors. Mufti et al. (2010) reported that when using AI to breeding animals the aim should be to achieve (40 to 70%) conception rate. There was no significant effect of housing conditions on conception rate. However conception rate was higher in animals with loose barn conditions (75%) while Tie stall housing conditions had conception rate of 71% (Table 1). The conception rate was higher in high feeding system (77%), while 70% of low feeding system had successful conception rate (Figure 3.1B: Table 3.1). The conception rate was better in autumn season 73% while it was 66% in spring season (Table 1). Size of heard affects conception rate, lower the size of heard higher the conception rate, herd size up to 5 animals had higher conception rate (86%), while size of herd 16 to 20 animals had (31%) conception rate (Table 1). One of the factors that can result in poor pregnancy rate is inaccurate estrous detection. This problem frequently occurs in large size herds. In small size herds animal keeper can easily detect heated cows because of contact and close monitoring. Many factors contribute to efficiency of artificial insemination. Factors that affect conception rate significantly in present study include size of herd, species, breed, milk production, age, body condition score, semen thawing, source of semen, site of semen deposit, effect of A.I technician, breed of bull/ox. The overall average conception rate observed in Tehsil Mansehra was (68%). One factor that resulted in poor pregnancy rate is inaccurate detection of estrous cycle.

EFFECT OF ATRAZINE ON TESTICULAR FUNCTION AND STRUCTURE IN YOUNG MALE FAYOUMI CHICKS

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The herbicide atrazine is a putative endocrine disruptor. This study evaluated the adverse effects of atrazine on the gross morphological features, testicular structure and function in the male Fayoumi chicks at the age of 13 weeks. For three weeks 10ppm and 20ppm doses were given orally in 0.1ml olive oil from 10 weeks till 13 weeks of age. Body weight gain, comb weight, liver weight, liver somatic index and left to right testis weight ratio showed no significant difference ($p>0.05$) of atrazine treated groups (10ppm and 20ppm) compared to control. 20ppm atrazine treatment caused significant ($p<0.05$) increase in the comb and wattle size compared to control and 10ppm atrazine treatment. A significant increase in the wattle size in 10ppm ($p<0.01$) and 20ppm ($p<0.001$) compared to control. Atrazine treatment caused significant ($p<0.01$) increase in testicular weight and gonadal somatic index. Atrazine treatment (10ppm and 20ppm) caused no significant difference in the length but the width of the left testis increase significantly in 10ppm ($p<0.05$) and 20ppm ($p<0.01$). A significant ($p<0.05$) decrease in testicular capsule thickness in atrazine treated groups (10ppm and 20ppm). There was dose dependent highly significant ($p<0.001$) increase in the seminiferous tubule diameter and significant ($p<0.01$) increase in the seminiferous epithelial height of atrazine treated groups (10ppm and 20ppm) compared to control. Atrazine treatment caused dose dependent significant ($p<0.001$) decrease in spermatogonia type A and leydig cell nuclear diameter than control. Atrazine treatment (10ppm and 20ppm) caused highly significant increase in the number of spermatogonia type B, primary spermatocytes and secondary spermatocytes. 20ppm atrazine treatments group caused significant increase in testosterone concentration than 10ppm atrazine treated and control group. Atrazine treatment caused no significant ($p>0.05$) increase in the estradiol concentration than control. Histomorphological studies revealed that atrazine treatment increased the interstitial space and enhance the spermatogenesis. Atrazine treatment caused the degeneration of tubule and enhance the spermatogenesis. Atrazine treatment caused the degeneration of tubules and no change in the morphology of spermatogenic cells, Leydig cell and sertoli cell. No mortality was observed in chicks, other morphological characters such as beak, phalanges, plumage, comb and wattle were appeared normal compared to control birds.

SYNTHESIS AND CHARACTERIZATION OF ORGANIC AND INORGANIC FORMS OF COBALT NANOPARTICLES: EFFECT ON GROWTH PERFORMANCE OF *TOR PUTITORA*

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Nanotechnology has diverse application in field of fisheries and aquaculture especially in aqua feed. Cobalt chloride (inorganic) and cobalt methionine (organic) nanoparticles (NPs) were prepared through physical (via ball mill) and chemical (through precipitation) methods and characterized by using scanning electron microscope SEM, electron dispersal spectrum EDS and X-rays diffraction methods. After that a 90 day feeding trial was conducted to study the comparative effect of graded level of CoCl₂-NPs and Co-Met-NPs supplemented diets on growth performance of *Tor putitora*. Mahseer fingerlings (average weight 6.5 g±0.23) were divided into two experimental sets'. Each set was further divided into six experimental groups, each in triplicate and fed CoCl₂-NPs and Co-Met-NPs (in triplicate) having dosage level of 0.5-3mg/kg each. Along with that one common control group of fish was fed basal diet, devoid of supplementation. Results indicated positive correlation ($R^2=0.9643$) in weight gain (%WG) and feed conversion efficiency ($R^2=0.9647$) in groups of fish fed CoCl₂-NPs up to 1.5mg/kg supplementation level, while further increased significantly ($P<0.05$) lower the %WG as compared to control group ($R^2=0.6164$). However, comparative to that, fish fed Co-Met-NPs as feed supplement showed significant ($P<0.005$) positive correlation in %WG ($R^2=0.969$) and FCE ($R^2=0.9518$) up to maximum supplementation level i.e. 3mg/kg in diet. Present study reveals the higher efficiency of Co-Met-NPs and suggests the replacement with CoCl₂ in mineral prefix mixture for obtaining better growth performance of fish.

DIETARY PROTEIN REQUIREMENT OF FRY OF *LABEO ROHITA*, *CATLA CATLA* AND THEIR INTERGENERIC HYBRIDS

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The present study was designed with the aim to investigate the appropriate dietary protein requirement of advanced fry of Thaila-Rohu Intergeneric hybrids compared with their parental species. Experiment was conducted in triplicate. Four group were classified as G1 (*Catla catla*), G2 (*Labeo rohita*), G3 (*C. catla* ♀ hybrid) and G4 (*L. rohita* ♀ hybrid). Advanced fry of each group were reared on three different crude protein level, i.e., 35%, 40% and 45%. After two month of feeding trial, analysis of growth performance, immunological indices, protein digestibility and challenge test were conducted. Two-way ANOVA indicated significant difference within and between groups with respect to all parameters. Results of growth performance (weight gain (%), SGR), immunological indices, i.e., Lysozyme and respiratory burst activity, AST activity, Activity of intestinal enzymes (amylase, protease and cellulase), metabolic enzymes (Alanine aminotransferase and aspartate aminotransferase) and mortality (%) after challenge with pathogen, *aeromonas hydrophilia* indicated the best performance of all groups when fed 40% CP diet followed by 35%CP diet, while poor performance of all groups observed at 45% CP diet. On the basis of results, it seems that during early rearing, *L. rohita*, *C. catla* and their intergeneric hybrids have similar 40% dietary requirement of protein.

COMPARATIVE INNATE IMMUNITY AND EXPRESSION OF IMMUNE RELATED GENES IN *CATLA CATLA*, *LABEO ROHITA* AND INTERGENERIC HYBRIDS

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The study was designed to evaluate the immunological indices as well of expression of immune response related genes in intergeneric hybrids of *C. catla* × *L. rohita* with their parent species. Fingerlings of hybrids and

parental fish species after tagging were stocked and reared for three month in the earthen communal pond. After the completion of experimental period, result indicated significantly higher levels of total Protein, Immunoglobulin, Lysozyme activity, Phagocytic activity %, Phagocytic index and Respiratory Burst activity in Catla♀ hybrid followed by Rohu ♀ hybrid while lowest values of all parameters were in *C. catla*. Furthermore, immune related genes showed variable expression in liver and muscle of all groups of fish, as in liver lysozyme G expression did not showed any significant difference in different groups while lysozyme C showed considerably higher expression in *Catla catla* followed by Catla♀ hybrid as compared *L. rohita* and rohu♀ hybrid. Furthermore, TNF alpha in liver of *L. rohita* and *C. catla* showed statistically similar and significantly higher expression as compared to both hybrids. Moreover, lysozyme C, G and TNF alpha showed up-regulated expression in muscle of both hybrids as compared to parental species. The significant improved status of immunological indices and up regulation of lysozyme C in liver and lysozyme C, G and TNF alpha in muscle of hybrids especially Catla♀ hybrid, indicating high immune-competency and suggesting its introduction in aquaculture system for gaining more production per acre.

CORRELATION OF LIVER ENZYMES WITH SEVERITY OF METABOLIC DISORDER IN OBESE FEMALES

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Obesity set a root of many metabolic disorders including diabetes mellitus, hypertension and infertility. Excessive BMI develop deranged liver enzymes leading top non-alcoholic fatty liver disease and ultimately fatty liver/cirrhosis. The study designed to evaluate the extent of altered liver enzymes that are linked with severity of metabolic disorders. Severity of diabetes and hypertension in obese females is strongly correlated with liver enzymes ALT and GGT and GGT is strongly enhanced in diabetic hypertensive obese females as well. Thus, raised GGT levels might serve as biomarker for diabetes and hypertension. Hepatic enzymes in obese infertile females however, remained within normal range and have no association with disease onset.

ANTIDIABETIC TREATMENT OF ALLOXAN INDUCED RABBITS

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The present research study was focused on the anti-diabetic treatment of alloxan induced rabbits by the use of synbiotic yoghurt. In this study rabbits were used as experimental model, diabetes was induced by alloxan injection on fasting condition. Then treatment diet was given to them which was supplemented with synbiotic yoghurt (0%, 2%, 4% and 6%) (combination of pro-and prebiotics) for a time period of four weeks (28 days). Then during the treatment duration their blood was assessed for the glucose, serum creatinine and urea levels. After induction of diabetes there was a sudden decline in weight of the rabbits and again the body weight was improved with oral administration of the synbiotic yoghurt. The lowest glucose level was observed in blood of rabbits after 4 weeks (28 days) of treatment with a mean decline from 239.50 mg/dl to 89.50 mg/. There was observed a significant change in serum creatinine level from 2.13 mg/dl to 0.99 mg/dl at the start of the experiments in all the treatments. The maximum reduction in urea level was observed after 4 weeks (28 days) in the rabbits fed on food supplemented with 6% synbiotic yoghurt with reduction from 81.47 mg/dl to 23.14 mg/dl. On the basis of these results it was concluded that the synbiotic yoghurt developed by the combination of prebiotics (fructo-oligosaccharide and isomalto-oligosaccharide) and probiotics (*Lactobacillus acidophilus*) may possessed significant potential for the management and treatment of diabetes.

EFFECT OF DIFFERENT CONCENTRATIONS OF FRUCTOSE AND GLYCEROL IN TRIS CITRIC ACID EXTENDER ON POST THAW QUALITY AND FERTILITY OF BUFFALO BULL SPERMATOZOA

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Fructose is considering as a vital energy source for metabolic events occurring naturally in the seminal plasma of buffalo spermatozoa. The aim of the present study was to explore the effect of different concentrations of fructose and glycerol in tris citric acid extender on post thaw quality and in vivo fertility of buffalo spermatozoa. Semen was collected from three bulls with a gap of four days through artificial vagina (42 °). Two ejaculates were collected from each bull per collection day and were evaluated initially for consistency, volume, motility, concentration, followed by dilution in five extenders (n= 5 aliquots, T1= F0.1%G7= Fructose 0.1 % glycerol 7 %; treatment 1, T2= F0.2%G7= Fructose 0.2 %, glycerol 7 %; treatment 2, T3= F0.4%G 6.5= Fructose 0.4 % glycerol 6.5%; treatment 3, T4= F0.8%G6= Fructose 0.8 %, glycerol 6 %; treatment 4, T5= F1.0%G5= Fructose 1 %, glycerol 5 %; supplementation). The experiment was replicated four times (n = 4). Data was assessed with analysis of variance. The results showed that percent progressive motility, plasma membrane integrity and supra-vital plasma membrane integrity of spermatozoa was significantly higher (P < 0.05) in extender supplemented with T5 than T1 and T2. Sperm hypo-resistivity, acrosome integrity and DNA integrity were significantly higher in extender supplemented with T5 than T1. Moreover, sperm in vitro quality was significantly higher in T5 than T1 during 30 and 60 min of incubation at 37°C. Sperm in vivo fertility was significantly higher in extenders supplemented with T5 (57.26%) as compared to T1 (41.32%). It is concluded that extender supplemented with T5 improved post thaw semen quality and in vivo fertility of buffalo bull.

SYNTHETIC AND NATURAL HORMONES (OVAPRIM, HCG AND HMG) SEPARATELY AND IN COMBINATION INDUCED SPAWNING IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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This study was carried out to investigate artificial breeding in female *Clarias gariepinus* through injection of synthetic hormones separately and in combination. The study was carried out 1st time in Muzaffargarh, Punjab, Pakistan. The stock of 2000 fish was imported from Thailand and raised at local fish hatchery in Muzaffargarh. After identification of male and female fish total 48 (24:24 / Male:Female) equally weighted fishes were selected for this study. All the males were injected with same concentration of ovaprim @ 0.3 mg/kg body weight to get milt. Females were injected with different hormones by dividing them into groups as A, B, C, D, E and F. Group A was control group and received no dose. Other groups received as B, Ovaprim (0.5); C, hCG (0.5); D, hMG (0.5); E, Ovaprim+ hCG (0.3+0.5); F, Ovaprim+ hMG (0.3+0.5). incubation Period (Hours), fertilization Rate (%), survival Rate (%), hatching (%), deformed larvae (%), fecundity (Nos), total egg weight (Grams), spawning success (%) and latency period (Hours) were studied in this experiment. A combination of Ovaprim+ Hcg (0.3+0.5) indicates the preminent results and economically beneficial for induced breeding in *Clarias gariepinus*.

ASSOCIATION OF MANAGEMENT AND TOPOGRAPHY WITH THE REPRODUCTIVE PERFORMANCE OF AZIKHELI BUFFALO IN NORTHERN PAKISTAN

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The Azikheli buffalo is native breed to the Hindukush Mountains of Northern Pakistan. These buffalo are kept in its home tract by different social groups (landowners, Gujars and tenants) under diverse topographic conditions (hill slopes, undulating areas and valley bottoms). Here we attempted to explore the management effect of social groups and topographic setting on the key reproductive traits of these local buffalo breed. The outcome of present findings is crucial to identify the probable habitat and management style that can host an indigenous buffalo breed conservation programme with success. Data was collected through a structured questionnaire from a total of 225 households representing the social groups and topographic zones aforementioned and was analyzed through a one-way analysis of variance. The current study demonstrated that social groups have no significant effect on reproductive traits studied except for postpartum anoestrus interval which was short ($P < 0.05$) for the buffaloes reared by tenants than by landowners. Azikheli buffalo reaches puberty earlier at valley bottom ($P < 0.01$) than other zones and has longer postpartum anoestrus interval at hill slope ($P < 0.05$) than undulating zone. The better overall first service conception (62.69%), number of services per conception (1.53 ± 0.06) and calving interval (480.62 ± 7.30 days) than other buffalo breeds under a variety of management conditions indicate a genotype-environment adaptability of the breed, and warrants its conservation. Gujars with larger Azikheli herds and having higher proportion of breeding bulls should be the primary recipients of a conservation programme.

IN-VIVO INDUCTION OF MICRONUCLEAR ABNORMALITIES IN *OREOCHROMIS NILOTICUS* UNDER BINARY METAL MIXTURES EXPOSURE OF LEAD, CADMIUM AND COBALT

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The aquatic ecosystem is final receiver of contaminants from untreated effluents, agricultural, industry, domestic and urban sectors. Among different contaminants metals are considered to be most important because of their non-biodegradable nature which causes them to get amassed in the organs of aquatic animals and come to be lethal due to their mutagenic nature. Keeping in view the nature of metals, experiment was planned to determine the dose dependent genotoxicity in terms of nuclear abnormalities in peripheral blood erythrocytes of *Oreochromis niloticus* under sublethal exposure of binary metals mixtures (Pb-Cd, Pb-Co and Cd-Co) by using the micronucleus assay. Present experiment was conducted under constant laboratory conditions, extremely limited efforts have been made to explore the genotoxic potential of metals in the form of mixtures. In first step, the 96-hr LC₅₀ of selected mixtures to fish were determined in a static bioassay system. Afterward, fish were exposed to sub-lethal doses viz. 1/3rd, 1/4th, 1/5th and 1/6th of 96-hr LC₅₀ of mixtures along with control group for the duration of 15 days. Fish blood was sampled after 15 days for preparation of micronucleus slides to observe the induction of nuclear abnormalities in the erythrocytic nucleus of treated and control fish. Among various concentrations, an exposure of 1/3rd LC₅₀ of Pb-Cd and Pb-Co mixture to fish induced significantly higher micronuclei frequency while same was observed lower in the control group. Under Cd-Co exposure, the frequency of micronuclei and other nuclear abnormalities varied significantly ($p < 0.05$) due to sub lethal concentrations that followed the order: 1/3rd > 1/4th > 1/5th > 1/6th > 1/7th > control. All selected metals mixtures gave statistically significant ($p < 0.05$) dose dependent nuclear abnormalities under

various sublethal concentrations. Acute toxicity tests are among the first step in determining the water quality condition for fish as these test gave toxicant concentration (LC50) that cause fish mortality even at short durations. In aquatic environment, metals are actually present in the form of mixtures; therefore, it was a dire need to evaluate the effects of metals mixtures on fish health.

EFFECTS OF DIFFERENT DIETARY LEVELS OF LYSINE ON GROWTH PERFORMANCE, CARCASS AND MORPHOMETRIC CHARACTERISTICS IN *CYPRINUS CARPIO*

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The objectives of the study were to determine the effects of various levels of dietary lysine on growth performance, carcass characteristics, body yield and morphometric characteristics in *Cyprinus carpio*. It was hypothesized that the greater levels of dietary lysine, than *Cyprinus carpio* requirements may improve growth performance, carcass characteristics, body yield and morphometric characteristics in *Cyprinus carpio*. In total, 240 fish (on an average 150gm body weight) were obtained from the Government Fish Hatchery and provided four isonitrogenous [32.5% crude protein (CP) and isocaloric (2840 kcal/kg)] dietary treatments with four replicates containing 15 fish each. The control diet (D1) contained 2.20% lysine, whereas the three experimental diets had graded levels of lysine (2.31% (D2), 2.51% (D3) and 2.67% (D4)). The fish were stocked in an indoor circular fiber glass fish tank (450 L capacity) for a period of 120 days. The results indicated that there was a linear improvement ($P < 0.05$) in growth performance with increasing the dietary lysine levels. Diet D3 (2.51% Lysine (10% supplemented)) resulted in an optimal performance, compared with the other dietary treatments, with 8% greater feed intake, 10.5% more body weight gain and an 11.8% better feed conversion ratio with highest specific growth rate compared with those fed the control diet (D1). Fish consuming D 3 had 6.8% greater dressed weight, 5.2% lower visceral weight and 7.4% greater fillet yield compared with those fed the control diet. An increase of 6.5 % in body length and 7.8% in standard length was observed in fish consuming diet D3 compared with those fed the control diet (D1). In conclusion, the improved growth performance, better carcass yield and higher morphometric relations in fish fed 10% extra dietary lysine (D3) indicated that higher dietary lysine improves the mentioned parameters in fish. Lysine supplementation in fish diets is, therefore, beneficial for economical and sustainable aquaculture production.

EVALUATION OF PHYSIOLOGICAL, BIOCHEMICAL AND OXIDATIVE STRESS LEVEL IN PREGNANCY INDUCED HYPERTENSION PATIENTS FROM LAHORE-PAKISTAN

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Hypertensive disorder of pregnancy, for example, pre-eclampsia (PE) and pregnancy-initiated hypertension (PIH) are a noteworthy reason for maternal morbidity. The rate of PE remains at 3-10% globally. PE was characterized according to the report issued by the Working Group of National High Blood Pressure Education Program on High Blood Pressure during pregnancy. Venous blood sample (5.0 ml) of 60 patients of PIH and 50 blood sample of healthy individuals was taken in clotted gel vial from Mayo Hospital and Jinnah Hospital Lahore. Biochemical components such as Vitamin A, Vitamin C, Vitamin E, MDA, CAT, GSH were SOD were estimated. The results revealed high serum level of MDA in PIH patients (14.21 ± 0.16) than control persons (3.28 ± 0.27). GSH level was decreased significantly (0.58) in PIH patients against control individuals (6.41) whereas Catalase activity was increased (6.92) in PIH patients when compared with the healthy persons (4.9). Likewise, SOD was increased significantly (13.06) in PIH patients as compared to control ones (2.17). The Vitamin E (0.56) in pregnant patients was drastically decreased in contrast to the control members (4.43). Findings of the present study clearly manifested that imbalance in the concentrations of oxidative stress markers are probably associated with PE than in

PIH compared to normal pregnancy. Imbalance in biomarkers and its factors depicted by an increase production of oxidants in caring for pregnant women with hypertension, it is important to differentiate among chronic hypertension, gestational hypertension, and PE.

VITAMIN C IMPROVE BIOCHEMICAL PROFILE OF PATIENT WITH TUBERCULOSIS INFECTION

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Tuberculosis (TB) is the second common cause of death. For to combat the disease like Tuberculosis (TB) role of ascorbic acid is very important as an antioxidant, autoimmunity and defensive against pathogens. Present study was carried out to find the effect of tuberculosis infection on Vitamin C status and bio- parameters in patients of tuberculosis. Ascorbic Acid level and other biochemical parameters were measured by using different biochemical techniques. Biochemical parameters which showed significantly ($p < 0.005$) low values included Vitamin C (0.24 ± 0.01 mg/dl), lipid profile total cholesterol (124.94 ± 0.80 g/dl), HDL Cholesterol (34.50 ± 0.40 g/dl), LDL Cholesterol (71.28 ± 0.61 g/dl), VLDL cholesterol (19.78 ± 0.17 g/dl) and triglycerides (99.88 ± 0.81 g/dl) and some serum proteins including total proteins (6.75 ± 0.05 g/dl) and albumin (2.78 ± 0.03 g/dl). Globulin level (3.97 ± 0.04 g/dl) was increased significantly in patients when compared with control subjects. These findings showed effect of tuberculous infection on bio chemical parameters. Due to infection these parameters are deranged thus cause complication.

EVALUATING THE SYNERGISTIC EFFECT OF NATURAL AND SYNTHETIC AGENTS ON WOUND HEALING

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Skin burns are cause by many factors like heat, electricity, chemical burn or radiation burn. The burns are treating naturally by different products like medicinal, herbal etc. Synthetically different medicines, growth factors, hormones and enzymes are using for the treatment of burns. Burn healing comprises of four stages: Hemostasis, Inflammation, Proliferation, remodeling. There are different local and systemic factors that effect on healing process. There are different types of dressings for treating the burn wounds. Burn is classified in different types on the basis of its thickness like superficial burn and partial burn. In this research work I shall evaluate the combine effect of natural and synthetic agents in the burn skin healing. Extract of *Moringa* leaves, as this part of plant effective in healing, potato peels that have been used in dressing and Curcumin will take as natural agents and boric acid, Povidone iodine and hydrogen peroxide as an anti-microbial will take as synthetic agent. The extract of *Moringa* leaves will collect by simple method which involves grinding, concentrating and filtration. Curcumin will extract from turmeric by solvent extraction method. PRP gel will formulate by above these agents. Burn will create on mouse and then PRP gel will apply topically on burn area till it heals. The combine effect of natural and synthetic agents will increase rate of healing, protect the burn wound from bacterial and fungal infection, and decrease the scar formation area.

NANOPARTICLES: SYNTHESIS AND APPLICATION

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Nanotechnology has the ability to transfigure the present technologies for the betterment of mankind. Nanotechnology has definite solutions to solve all the problems related to agriculture like pest control by chemicals.

Different types of nanoparticles can be synthesized by different methods based on their applications. Due to their small size, nanoparticles have a great interest in the coming or present era as they suited in all applications. They also applicable for pest control management, due to their small size they are used as pesticides to oversight the usage of chemical or other harmful pesticides sprays. Nanotechnology will be useful in the agriculture because they are eco-friendly and have an excellent impact on crops production. On the other hand, they have also some hazard impact on ecosystem due to their extra usage. Agriculture will be protected by the usage of nanotechnology because they will reduce the usage of chemical sprays. Nanodevices and nano products are made by nanotechnology and they are used to deliver pesticides and growth regulators for the betterment of crops, condition of soil and it also control the attack of pest on plants. Nanotechnology gives the impression of being bright in the future in different sectors.

PREPARATION AND ANALYSIS OF SYNTHETIC FOOD PRODUCT FOR CURE OF DIABETES

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Functional food components are most beneficial components that can naturally found in different foods and also added as functional ingredients in the food such as carotenoids, dietary fiber, fatty acids, flavonoids, prebiotics, probiotics, vitamins and minerals. Some functional food components play a key role the health augmentation. The presently important bioactive compounds in foods either naturally or either added lead many scientists of different field to conduct many studies for establishing of scientific basis and validates the benefits of a particular food or components for the human health. Diabetes is disease in which the blood sugar level are too high and insulin is a hormone to give energy into cell. To normalize the glucose level by using natural indigenous sources such as ginger which has long been used as herbal medicine to treat various symptoms including vomiting, pain and cold symptoms and also contains anti-inflammatory, anti-diabetics and analgesic properties and amla is the richest source of vitamin C and also contains gallic acid, allergic acid and flavonoids. It possesses antioxidants, anti-hyperglycemic and anti-hyperlipidemic properties. Barley is a cereal crop enriched with high concentration of beta-glucan which is actively involved in reducing the cholesterol level and also regulates blood glucose level and insulin response in diabetes. Barley has high fiber content, adequate amount of mineral, antioxidant and vitamin. It has nutritional benefits which protect from diabetes and heart health. The objective of this study is to preparea synthetic product from indigenous sources for the cure of diabetes. The prepared product will be analyzed by physic-chemical and antioxidant analysis. The effect of prepared product will help to maintain the glucose level in diabetes. The animal trials will be performed to check the toxicityby using specific dosage of prepared product in subject and control group.

ELUCIDATION OF THE ROLE OF HISTONE PROTEINS IN THE DINOFLAGELLATE *ALEXANDRIUM PACIFICUM* DURING REGULATION OF ITS GROWTH

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Histone proteins package DNA in all eukaryotic organisms and play a key role in the regulation of gene expression. The exception is dinoflagellates, which have histone protein expression that is mostly reported to be below detectable levels. The motivation for this study was that the dinoflagellate *Alexandrium pacificum* is among the major species causing harmful algal blooms (HABs) that produce paralytic shellfish toxins. In the present study, transcriptomic sequence data from *A. pacificum* is analyzed, and the diversity of gene encoding histone proteins and their variants are characterized, with particular focus on their potential of posttranslational modifications. The expression quantification of these genes and proteins was performed using real time PCR and western blot analysis during the cell cycle stages and under different nutritional conditions. To our knowledge, this is the first report of

immunological detection of anti-histone-reactive proteins (H2B and H4) in any dinoflagellate species. We conclude that *A. pacificum* expresses multiple variants of histone, and protein sequences revealed both conservation and divergence with respect to other eukaryotes. The stable expression of histones during the cell cycle suggested that the histone genes in *A. pacificum* belonged to a replication-independent class and appeared to have a limited role in DNA packaging. The conservation of numerous post-translationally modified residues (histone code) of multiple histone variants and differential expression of histones under nutritionally enhanced conditions suggested their functional significance in dinoflagellates. However, we detected histone H2B protein only via mass spectrometry. Histone-like protein was identified as most abundant acid-soluble protein of the cells.

STUDIES ON EGG LAYING AND EGG PARAMETERS OF ANAS PLATYRHYNCHOS OF DISTRICT DADU SINDH, PAKISTAN

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Anas platyrhynchos is the most recognized waterfowl in the world with typical duck shaped body. These birds are hunted for food, eggs and meat and are good source of protein. They are important ornamental birds and kept for aesthetic purpose also domesticated. These birds were captured during the month of March 2017, *Anas platyrhynchos* was found to be an early breeder; egg laying started in the month of march and continued till June, April was found to be peak egg laying month. 15 nests were studied during present study. Mean egg weight was 48.27gm, while as the average egg size was 56.42 x 40.55mm (± 2.34 x ± 1.19). Average clutch size was found to be 8.27 (± 2.23).

FRAXINUS XANTHOXYLOIDES EXTRACT ALLEVIATES CISPLATIN INDUCED REPRODUCTIVE TOXICITY IN MALE ALBINO RATS

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Cisplatin is the most effective chemotherapeutic antitumor drug, which is able to produce reactive oxygen species (ROS). Cisplatin has been observed to induce reproductive toxicity in animals. Moreover, Cisplatin restrain the action of antioxidant enzymes in the body. In this study, the dose dependent curative potential of *Fraxinus xanthoxyloides* extract on Cisplatin induced toxicity of reproductive organs in albino rats was evaluated. This study was conducted on male albino rats that were taken from the animal house of University of Agriculture, Faisalabad. The rats were divided into eight equal groups. Group one: Control; Group two: Vehicle control; Group three: Cisplatin (10 mg/kg); Group four: Cisplatin (10 mg/kg) and Silymarin (100 mg/kg); Group five: Cisplatin (10 mg/kg) and *Fraxinus xanthoxyloides* (200 mg/kg); Group six: Cisplatin (10 mg/kg) and *Fraxinus xanthoxyloides* (400 mg/kg); Group seven: *Fraxinus xanthoxyloides* (200 mg/kg) and Group eight: *Fraxinus xanthoxyloides* (400 mg/kg). Rats were slaughtered at the end of the experiment and blood was collected in EDTA tubes. Testes of rats were obtained. The parameters of this study were daily sperm production, estimation of antioxidant enzymes, hormonal analysis and histopathology. Cisplatin treatment also induced significant ($p < 0.05$) decrease in weight of rats, testicular weights, tunica albuginea height, seminiferous tubules diameter, seminiferous tubules epithelial height, testosterone concentration in testicular tissues and in plasma, daily sperm production, Catalase (CAT), Peroxidase (POD), Superoxide Dismutase (SOD), Glutathione reductase (GSR), spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids when compare it with other groups whereas Cisplatin treatment induced significant ($P < 0.05$) increase in interstitial space, tubular luminal diameter and the level of TBARS in Cisplatin treated group. The level of TBARS was decreased in *Fraxinus* treated groups. Our results indicated that Cisplatin exerted its toxic effects on testes and *Fraxinus xanthoxyloides* ameliorated toxic effects of Cisplatin on reproductive organs. Therefore, it was concluded that *Fraxinus xanthoxyloides* extract have potential efficacy to ameliorate Cisplatin induced toxicity in rat testes.

PREVALENCE OF MALNUTRITION AMONG CHILDREN UNDER FIVE YEAR OF AGE IN DISTRICT THARPARKAR, SINDH, PAKISTAN

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Malnutrition is the major cause of mortality in children. Several studies have suggested higher prevalence of malnutrition in underdeveloped countries particularly Pakistan. Number of factors has been associated with increasing malnutrition in children; these factors are food insecurity, poverty and infectious diseases. Malnutrition is common in all age groups particularly in children under five year of age. Tharparkar is the desert area with least agriculture, and increasing malnutrition. An epidemiological cross-sectional study is carried out in four Tehsils of District Tharparkar. A total of 301 children under five years age were randomly selected and assessed for malnutrition by measuring the middle upper arm circumference (MUAC), weight, height and head circumference. The purpose of this study is to investigate the prevalence of malnutrition in children under five years of age as well as to point the risk factors associated with malnutrition in District Tharparkar. Our data indicate higher prevalence of stunting (81.1% n=244), underweight (62.4% n=188), wasting (18% n=54) and prevalence of malnutrition according to MUAC is 56.1% n=169). The factors associated with malnutrition are drinking water, infectious diseases siblings. In conclusion higher prevalence of malnutrition was found in children under five of age in district Tharparkar. The present study demonstrates the alarming malnourished conditions in children under five years age in drought affected area of Pakistan that is Tharparkar. Overall girls are more severely malnourished than boys. Stunting has been found the highest in the world.

PREVALENCE OF METABOLIC SYNDROME IN PRE AND POSTMENOPAUSAL WORKING WOMEN IN HYDERABAD, PAKISTAN

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Increasing evidence suggest the high prevalence of metabolic syndrome (Mets) in Post-Menopausal Women. High prevalence of Mets is often associated with diabetes and cardio vascular diseases. Pakistan has high mortality rate due to Cardio vascular diseases. No extensive study regarding prevalence of Mets and its association with diseases has been carried out. The main purpose of this study is to show the prevalence of metabolic syndrome among post-menopausal working women. This will be the first study on Mets, which will give the participants awareness to prevent the cardio vascular diseases. Cross sectional study was carried out on 276 working women, randomly selected from degree colleges of Hyderabad region. The data was collected through interview based structured questionnaire. The healthy volunteers aged < 60 years of age were included in this study. All those who were on medication or have any known diseases were excluded from the study. Demographic information was achieved. Venous blood sample was collected in the morning time, while respondents were fasting. Serum was collected after centrifuging the samples; serum was kept at 4°C. Estimation of Triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol was carried out using Spectra XL fully automatic machine. Statistical analysis was carried out using SPSS 16. The ethical approval was obtained from the Ethical Review Committee, University of Sindh. Out of 276, overall prevalence of Mets was 46.37% (n=128), postmenopausal women had significantly higher prevalence of Mets 31.88%, ($P < 0.0001$) than premenopausal women 14.49%. According to the odd ratio, the postmenopausal women had significantly higher odds of hypertension (O.R = 7.7, $P < 0.0001$) than premenopausal women. No significant difference of odd ratio for Waist Circumference (O.R = 0.93), Fasting blood Glucose (O.R = 1.7) HDL (O.R = 0.66), Triglycerides (O.R = 1.34) was found between post-menopausal women and premenopausal women. Further postmenopausal women had significantly higher prevalence of all five components ($\chi^2 = 16.4$, $P < 0.0001$) than premenopausal women. In conclusion, postmenopausal women had significantly higher prevalence of Mets in post-menopausal women; post-menopausal women were significantly at higher risk of suffering from CVDs.

INDEPENDENT PERIAQUEDUCTAL GREY AND HYPOTHALAMIC CIRCUITS FOR FLIGHT RESPONSE AND ORIENTED BEHAVIOURS ELICITED BY INNATE FEAR

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The aim of this study was to investigate the role of dPAG and dorsomedial division of the ventromedial hypothalamic nucleus (VMHdm) in the organization of explosive and oriented behaviours, respectively. Male Wistar rats (n=7 per group) were used. A guide cannula was stereotactically implanted in the dPAG and VMHdm. Five days after surgery and three days after habituation of the animals in a polygonal arena with artificial burrow, central microinjections of 200nl of saline or cobalt chloride (CoCl₂) (1mM/0.2 uL) were performed into the dPAG followed by 200nl of NMDA (9nmol) given into the VMHdm. During microinjection, the animals were gently handled. Immediately after microinjection, exploratory behaviours, oriented defensive escape and explosive defensive escape behaviours were recorded for 10 minutes in a polygonal arena with artificial burrow. After experiment, perfusion and histology were done. One way ANOVA showed that the microinjection of CoCl₂ in dPAG abolished explosive escape (p<0.001), freezing (P<0.001) and alertness (P<0.05) (frequency and duration), as well as frequency of long jumps (P<0.001) and vertical jumps (P>0.05) elicited by stimulation of VMHdm with NMDA at 9nmol, compared to the control group and to saline into the dPAG followed by 9nmol NMDA in VMHDM-treated group. The same treatment also increased rearing frequency (p<0.001), rearing duration (p<0.001), escape to the burrow frequency (p<0.01) and duration (p<0.05) as well as increased the frequency of climbs (p<0.001) and jumps (p<0.001) to the burrow, compared to saline in dPAG followed by 9nmol NMDA in VMHDM treatment. Physiological saline pretreatment of the dPAG followed by 9nmol NMDA microinjections into the VMHDM decreased frequency and duration of the explosive escape (p<0.001), freezing (P<0.001) and alertness (P<0.05), as well as frequency of long jumps (P<0.001) and vertical jumps (P>0.05) elicited by stimulation of VMHDM with 9nmol NMDA, compared to the control control group and the treatment of the dPAG with CoCl₂ followed by microinjections of NMDA at 9nmol into the VMHDM. This treatment also decreased the rearing frequency (p<0.001), rearing duration (p<0.001), escape to the burrow frequency (p<0.01) and duration (p<0.05) as well as decreased the frequency of climbs (p<0.001) and jumps (p<0.001) to the burrow, compared to the pretreatment of the dPAG with CoCl₂ followed by the treatment of the VMHDM with NMDA at 9nmol (p<0.05 in all cases). These findings showed that the dPAG is independently responsible for fear-induced flight response. On the other hand, the VMHdm organises more oriented behaviours. The present results corroborate previous data, showing that lesions in the dPAG cease the responses evoked by hypothalamus stimulation.

RANDOM MUTAGENESIS OF *BACILLUS MEGATERIUM* FOR PRODUCTION OF LYSINE

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Bacillus megaterium is biotechnologically important microorganism which is used for the production of L-lysine. In present study wild strain of *B. megaterium* produced 0.51g/L Lysine in suitable and optimized fermentation medium. This wild strain was subjected to ultraviolet (UV) treatment. For this purpose bacterial strain was plated on nutrient agar plates and exposed to UV light for 10 min to 45 min, until 95% death rate was obtained. Out of thirty survivors, only two showed higher amount of L-lysine production as compared to wild strain. Survivor or mutant BM^{UV5} produced 0.61g/L L-lysine and BM^{UV26} showed 0.65g/L Lysine production. Wild strain was also subjected to chemical mutagenesis. For this purpose chemical mutagens *N*-ethyl-*N*-nitrosourea (ENU), ethyle methane sulfonate (EMS) and nitrous acid (NA) were used for different time intervals, until 95% death rate was observed on nutrient agar plates after incubation of 24 hrs to 72 hrs. About 150 survivors were obtained, out of these only seven showed higher amount of Lysine production as compared to wild strain of *B. megaterium*. Out of these seven mutants BM^{ENU15} and BM^{EMS22} were found to produce maximum 0.97 and 1.2 g/L of Lysine in suitable fermentation medium.

PREVALENCE OF CHRONIC COMPLICATIONS TYPE 2 DIABETES MELLITUS IN PATENTS FROM HYDERABAD AND JAMSHORO

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Diabetes mellitus DM is one of the most common diseases cause premature death. It is due to dysfunction of beta cells of pancreas which cause glucose level raise in body. It has 2 types i.e., Type-1 and Type-2 DM. In each year almost 1million people die because of DM and it is rapidly growing worldwide. Asia has the fastest growing number of patient with DM. In India, China, Pakistan and Japan having 33,23,9 and 7 million peoples are respectively with the diabetes mellitus. According to WHO, while the prevalence of diabetes in Pakistan is 8.6%, 11.1%, 13.9% and 52.4 % in the provinces of Baluchistan, NWFP, Sindh and Punjab respectively. Globally, T2DM has become one of the most important chronic public health problems. T2DM is a growing cause of mobility and mortality, because of cardiovascular disease and other chronic vascular complications. During the present study data was collected from indoor patient of all medical units of LUMHS Hospitals of Jamshoro and Hyderabad. From the month of 1st January to 30th June 2017. The subjects were interviewed through questionnaire and diagnose through Biochemical markers correlating with these complications. In the present study 140 patient include 60% male mean age 56.36 and 39.2% Females mean age 56.96 Among the 140 patient cardiovascular disease found in 21.1% male mean age 56.11 and 20% Female mean age 53.72, Cerebrovascular disease found in 34.1% male mean age 58.1 and 38.1% Female mean age 61.04 and Nephropathy found in 54.11% male mean age 57.3 and Female 60% mean age 58.42. This is the first time reported work from above said project.

7. TOXICOLOGY

ACUTE EFFECT OF BIFENTHRIN+CHLORPYRIFOS MIXTURE ON GLUTATHIONE-S-TRANSFERASE ACTIVITY IN FRESHWATER FISH, *CTENOPHARYNGODON IDELLA*

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Glutathione-S-transferases are cytosolic enzymes and very essential in biotransformation process. It is also important in the detoxification of electrophilic substance. In present study acute toxic effect of pesticides mixture (bifentherin+chlorpyrifos) on glutathione-S-transferase (GST) activity and total protein contents in different organs (gills, liver, brain, muscle, heart and kidney) of fish, *Ctenopharyngodon idella* was evaluated. The fish was exposed to 96-hr LC₅₀ concentration of bifentherin+chlorpyrifos mixture for 4-day and sampling was done at interval of 24-hr. The activity of GST was measured by using spectrophotometer. The results indicated that the exposure of pesticides mixture significantly increased the GST activity in all observed organs of fish with increasing the duration of exposure while total protein contents were decreased.

ASSESSMENT OF NUCLEAR ABNORMALITIES IN PERIPHERAL ERYTHROCYTES OF CARNIVOROUS FISH, *WALLAGO ATTU* EXPOSED TO LEAD

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Heavy metals that are present in the water systems ingested by the aquatic organisms and these pollutants altered their genetic makeup. Metals have ability to generate reactive oxygen species that would cause the oxidative of DNA. Micronucleus test has been generally used for the evaluation of biological impact of aquatic pollutants on genotoxic damage in fish. Therefore, current research work was conducted to assess the nuclear abnormalities in peripheral erythrocytes of lead exposed *Wallago attu* by using micronuclei assay. Fish were exposed to the various sub-lethal concentrations (1/3rd, 1/4th, 1/5th and 1/7th of LC₅₀) of lead for 21 days. Blood sample from caudal vein of fish was collected after 7-day interval to see the micronuclei and deformed nuclei in peripheral erythrocytes of fish. Results showed that all test concentrations significantly induced the micronuclei and deformed nuclei in peripheral erythrocytes of fish. Maximum nuclear abnormalities in peripheral erythrocytes of *W. attu* were observed in 1/3rd of LC₅₀ concentration followed by the order: 1/4th > 1/5th > 1/7th.

MICRONUCLEI ASSAY: A SUITABLE TOOL FOR EVALUATING THE HEAVY METALS (CR+CO) INDUCED GENOTOXICITY IN FISH *LABEO ROHITA*

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The present research was conducted to assess the genotoxic potential of cobalt (Co) and chromium (Cr) mixture to fish *Labeo rohita* by using micronuclei assay. Fish were exposed to the four sub-lethal concentrations (2/3rd, 1/3rd,

1/4th and 1/5th of LC₅₀) of Cr+Cr mixture for 28 days and sampling was done after 7 days interval. A group (n=10) of fish were also kept in clean water (negative control=NC) and cyclophosphamid (positive control=PC), separately. Blood from caudal vein of fish was collected to see the micronuclei and de-shape nuclei. Results showed that all concentrations induced micronuclei and de-shaped nuclei in peripheral erythrocytes of *L. rohita*. Exposure of Cr+Co mixture at 2/3rd LC₅₀ concentration induced significantly higher mean abnormalities followed by that of 1/3rd, 1/4th, 1/5th, PC and NC. However the result of de-shaped nuclei showed minor difference. The frequency of de-shaped nuclei higher due to 2/3rd concentration of mixture followed by the order: 1/3rd > 1/4th > PC > 1/5th > NC. The mean frequency of micronuclei and de-shaped nuclei varied with the duration of exposure as 28 > 21 > 14 > 7 days. This study concluded that metals present in mixture in aquatic environment can induce DNA damage in fish.

OXIDATIVE ENZYME DYNAMICS AND GENOTOXICITY IN CATLA INDUCED BY BIFENTHRIN+CHLORPYRIFOS MIXTURE

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A semi-static experiment was performed to determine the acute toxicity in term of 96-hr LC₅₀ and lethal concentration of technical grade pesticides mixture (bifenthrin+chlorpyrifos) to Indian carp (*Catla catla*). Furthermore, the effect of bifenthrin+chlorpyrifos mixture on the glutathione S-transferase activity in various tissues (brain, liver, gills, kidney, heart and muscle) of *C. catla* after 24, 48 72 and 96-hr was studied. Genotoxic potential of bifenthrin+chlorpyrifos mixture in peripheral erythrocytes of fish was confirmed through micronucleus assay. The mean 96-hr LC₅₀ and lethal values were estimated as 2.09 and 3.44 µg/L⁻¹, respectively. The number of dead fish was significantly increased as the concentration of bifenthrin+chlorpyrifos mixture increased. Binary mixture exposure significantly increased the glutathione S-transferase activity in time dependent manner in all selected organs of the exposed fish. Time duration dependent increase in micronuclei frequency and other nuclear abnormalities (binucleated nuclei, dumbbell nuclei, blebbed nuclei, notched nuclei and de-shaped nuclei) were observed due to BIF+CPF mixture exposure.

STUDIES ON THE HISTOPATHOLOGICAL CHANGES IN LIVER AND GILLS OF THE FISH OREOCHROMIS NILOTICUS EXPOSED TO HEAVY METAL (LEAD ACETATE)

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This study was carried out on the liver of fish, *Oreochromis niloticus* to see the toxic effect of Lead acetate on liver. In this context, two concentrations of lead acetate 30mg and 40mg per liter water were applied on the fish maintained in the aquaria for a period of 30 days. After the completion of the experimental period, the fish (control and experimental) were sacrificed and processed for the histopathological studies. According to the histopathological studies, the effect of the tested heavy metal at both the concentrations was noticed, but the drastic effect was noted at the higher concentration (40mg./lit.). The changes were noted in all the areas of the liver i.e. in the parenchyma as well as in the stroma. The hepato cords and the hepatocytes in the first instance decreased in size followed by Picnosis, Necrosis and hepatoma in the fish exposed to the higher concentration (40mg./lit.). It is concluded that lead acetate is very injurious to the health of the aquatic life specially fish which is the source of proteinous diet for human being.

GENOTOXIC POTENTIAL OF AGROCHEMICALS ON *PERNA VIRIDIS***Nafisa Shoaib* and Aisha Majid Ali***Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270*

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Genotoxicity is considered as one of the endpoints in assessing toxic effect of pollution. In the present study genotoxic effects of pesticides in the *Perna viridis* have been evaluated by the induction of micronuclei (MN). The aim of the present study is to assess the MN frequency in the haemolymph of green mussel (*Perna viridis*) after exposure to different concentrations of organophosphate pesticides (chlorpyrifos, malathion), synthetic pyrethroid pesticide (cypermethrin, lambda-cyhalothrin) and herbicide (bucril). The bivalves were exposed to different concentrations (0.5ppm, 1ppm and 1.5ppm) of test pesticides in a static system. The MN frequencies of all the pesticide treated mussel groups increased significantly ($p < 0.05$) until the end of the exposure period as compare to control. The highest MN frequencies were recorded after cypermethrin exposure on day 12 (7, 8.5 and 11% for 0.5 ppm, 1 ppm and 1.5 ppm concentrations respectively) in haemolymph. However the lowest MN frequencies were recorded after bucril exposure (3.5, 3.5 and 5% for 0.5 ppm, 1 ppm and 1.5 ppm concentrations respectively) in haemolymph. The genotoxicity of pesticides in haemolymph of *Perna viridis* in this study is found to be in the order of cypermethrin > chlorpyrifos, > malathion, > lambda-cyhalothrin > bucril.

DETERMINATION AND IMPACT OF HEAVY METALS IN COMMON MAYNA (*ACRIDOTHERES TRISTIS*) OF DISTRICT FAISALABAD**Rimsha Naz¹, Irfan Baboo², Khalid Javed Iqbal³, Sana Malik¹, Naunain Mehmood⁴ and Iqra Rana⁵ sajid Abdullah¹**¹*Department of Zoology, University of Agriculture, Faisalabad*²*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*³*Department of Zoology, TheIslamia University Bahawalpur, Bahawalpur*⁴*Department of Biological Sciences, University of Sargodha*⁵*Department of biotechnology, university of Agriculture, Faisalabad*

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Determination of heavy metals is important since they influence different bird species and also have a great impact on environment. The study purpose was to find out the impact of heavy metals on urban and rural species of the common myna. This study was planned with the objective to select different male and female species of common myna from different areas of Faisalabaddistrict. Effect of selected heavy metals such as Mn, Zn, Pb, Cu, Cd and Co on different organs i.e. lungs, feathers, bones, muscles and heart was evaluated. The concentrations of selected heavy metals were ascertained by using atomic absorption spectrometry (ASS). The high amount of metals was found in different concentrations. Statistically, analyze the results and standard mean deviation was also implemented. The findings of this study highlight that despite restricted emission, heavy metals are found in environment, causing great health hazards in species of avian faunas and also on environment. Feathers of birds showed that, they can be used as bio-monitoring tools.

PROTECTIVE POTENTIAL OF *ALLIUM SATIVUM* EXTRACT ON SODIUM ARSENATE INDUCED TERATOGENICITY IN *MUS MUSCULUS***Madeeha Arshad*, Asmatullah and Chaman Ara***Department of Zoology, University of the Punjab, Lahore, Pakistan*

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Allium sativum (garlic) has antihypertensive, anticarcinogenic, anti-inflammatory, anti-fungal and anti-aging properties. It contains 33 organo-sulphur compounds, an active component allicin and more importantly contain

selenium which can antagonize arsenic. Naturally occurring arsenic is a ubiquitous environmental toxicant known to contaminate water supplies around the world as well as in Pakistan, primarily in the form of arsenate and arsenite. Exposure to high levels of arsenic may cause carcinogenicity, mutagenicity and teratogenicity. Present study was designed to explore the teratogenicity of sodium arsenate and to check the protective potential of garlic extract. Different doses of sodium arsenate, 0.00, 75, 37.5 and 18.75 µg/g B.W. were administered to gravid mothers (*Mus musculus*) on days 6-12 of gestation, accompanied by three other groups, which were treated with 125 µg/g B.W. garlic extract together with 75, 37.5 and 18.25 µg/g B.W. of sodium arsenate and fetuses were recovered on day 18 of gestation. Fetuses from dose groups 75, 37.5 and 18.75 µg/g B.W. showed certain organs disproportions like micromelia and hyperextensions, hygromas, skin hemorrhage, short tail, and distorted axis, along with fetal resorptions while fetuses belonging to 75, 37.5 and 18.75 µg/g B.W. and 125 µg/g B.W. appeared almost normal. Body weight of fetuses were reduced significantly ($P < 0.05$) with increase in concentration of dose. Morphometrically, head circumference, eye circumference, tail length, forelimb and hind limb size showed dose dependent decrease in fetuses obtained from sodium arsenate treated groups but groups treated with garlic extract along with sodium arsenate did not showed any significant difference in morphometric parameters. At histological level spina bifida, anophthalmia and certain other defects which were decreased by the administration of garlic extract. From results it can be deduced that garlic has the ability to overcome the teratogenic effects caused by arsenate.

HISTOPATHOLOGICAL LESIONS CAUSED BY ASPARTAME IN VITAL ORGANS OF MICE AND ATTENUATION BY SESAME OIL

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Aspartame is one of the most widely used artificial sweetener. It is currently used in well-known brands of foods and beverages. There is much controversy regarding its safety. The present study was designed to investigate the probable hepatotoxic and nephrotoxic effects associated with aspartame and attenuation by sesame oil in mice. The study comprised of four groups of forty male albino mice. First group was control (C), second was dose (D) treated with aspartame 40µg/g body weight of mice, third group was treated with sesame oil following aspartame administration and served as dose plus antidote group (D+A). While, fourth group was retained as vehicle control group (V.C) and given only sesame oil. The experiment was carried out for consecutive sixty days. At the day sixty-one, mice were anesthetized with chloroform and sacrificed. Required organs were excised and preserved for this multi-parametric study. The observations revealed that aspartame increased the average body weight of mice and the average weight and size of liver and kidney. Biochemical parameters were also altered after exposure to aspartame in a way that there was significant elevation in serum ALT, AST, ALP, bilirubin and decrease in serum albumin indicating liver malfunction. Urea and creatinine levels also changed as compared to controls, indicating renal impairment. Histopathological examination of liver and kidneys revealed disturbed hepatic and renal architecture and various histopathological anomalies following aspartame administration were hepatocellular injuries, hepatic congestion, vaculations, anisocytosis, renal hemorrhage, increased bowman's space, dilated renal tubules, partial loss of brush border of renal tubules, mesingio-proliferative glomerulus, glomerular atresia and peritubular nephritis. However morphological, morphometric, biochemical and histopathological abnormalities were markedly improved and reversed to a great extent on co-administration of aspartame with sesame oil. Therefore, it is concluded from current findings that aspartame has damaging effects on vital organs like kidney and liver, while sesame oil has curative role against aspartame induced hepato and nephrotoxicity.

ASSESSMENT OF ADVERSE EFFECTS OF CADMIUM AND MERCURY ON LIVER AND BLOOD OF DOMESTIC CHICKS AND PHARMACOLOGICAL INTERVENTION BY VITAMIN E

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The current research was undertaken to assess the adverse effects of cadmium and mercury on liver and blood of domestic chicks. Dose of cadmium chloride and mercuric chloride and vitamin E were diluted with distilled water and were given orally for thirty days. Doses given were as CdCl₂ 20.5 mg/kg body weight, HgCl₂ 4.5 mg/kg body weight and vitamin E 50 mg/kg body weight respectively. Blood samples were taken for analysis of hematological and biochemical parameters. Different biochemical and hematological parameters i.e. ALAT, ASAT, ALP, GPT, LDH, Glutathione, Hemoglobin, PCV, TEC, WBC count and MCHC were analyzed to estimate adverse effects on liver and blood. The results of this study revealed that in groups exposed to cadmium, mercury and their combination, biochemical parameters such as ALAT, ASAT, ALP, LDH, GPT were elevated significantly except glutathione which declined in comparison to control group with the passage of time till post treatment. Hematological parameters like Hb, PCV, MCHC and TEC decreased significantly while WBCs increased. While pretreatment with vitamin E significantly reversed the anomalies caused by these metals. Level of ASAT increased significantly till post treatment of Cd ($p \leq 0.001$), Hg ($p \leq 0.001$) and Cd+Hg ($p \leq 0.001$). Levels of ALAT ($p \leq 0.001$), ALP ($p \leq 0.001$), LDH ($p \leq 0.001$), GPT ($p \leq 0.001$) and Glutathione ($p \leq 0.001$) also showed highest significant differences as compared to control till post treatment of cadmium, mercury and their combination. On the other hand, hematological parameters were significantly declined except WBCs which were increased. Highest significant differences were shown i.e. Hb ($p \leq 0.001$), PCV ($p \leq 0.001$), MCHC ($p \leq 0.001$), TEC ($p \leq 0.001$) and WBC ($p \leq 0.001$) as compared to control group till post treatment of cadmium, mercury and their combination. When metal concentration in liver was analyzed it was observed that there was significant bioaccumulation of cadmium (42.33 ± 1.86 mg/kg body weight) and mercury (27.33 ± 2.33 mg/kg body weight) in liver as compared to control group (0 ± 0 mg/kg body weight). While in prevention groups it was Cd+VitE (18.00 ± 1.15 mg/kg body weight) and Hg+VitE (14.00 ± 2.89 mg/kg body weight) as compared to cadmium (42.33 ± 1.86 mg/kg body weight) and mercury (27.33 ± 2.33 mg/kg body weight). It was concluded that cadmium and mercury either separately or in combination have highly toxic effects on liver and blood parameters of chicks while vitamin E has synergetic effects against toxicity of aforementioned metals.

THE PROTECTIVE ROLE OF ASCORBIC ACID IN THE HEPATOTOXICITY OF CADMIUM AND MERCURY IN RABBITS

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Liver is one of the important sensitive organs which are usually exposed against the toxicity of mercury (Hg) and cadmium (Cd). The main purpose of this study was to evaluate the toxicological effects of individual and combined disclosure of Cd and Hg on biochemical parameters of liver, accumulation in liver as well as therapeutic role of vitamin C in rabbits (*Oryctolagus cuniculus*) against these metals. In current research, cadmium chloride (1.5 mg/kg), mercuric chloride (1.2 mg/kg) and vitamin C (150 mg/kg of body weight) were orally administered to treatment groups of the rabbits for 28 alternative days. Various biochemical parameters of liver such as lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), bilirubin, Alanine aminotransferase (ALAT), total

protein and gamma glutamyl transferase (GGT) were estimated using blood samples. Present study showed that body weight of all Cd and Hg treated rabbits did not significantly increase except vitamin C and control group. Some biochemical parameters like ASAT, ALAT, LDH, GGT, and Bilirubin were significantly elevated ($p \leq 0.001$) in individual Cd and Hg treatment groups, while level of total protein was found to be significantly decline. The effects of Cd and Hg in the presence of vitamin C on these biochemical parameters were less as compared to metals exposed groups. Similar results were found when rabbits were treated with co-administration of both metals and Vitamin C. Accumulation of Cd and Hg found to be higher in liver. However, chemoprevention and chemotreatment with vitamin C significantly ($P \leq 0.01$) minimize the toxicological effects of both metals but not regain the values similar to control group. This study provides awareness about the toxicity of both metals on biochemical parameters and accumulation in liver of rabbits as well as therapeutic role of vitamin C against these metals.

ANATASE TITANIUM DIOXIDE NANO PARTICLES MEDIATED TOXICITY INDUCED GENOTOXIC AND HEMATO-BIOCHEMICAL EFFECTS IN SPRAGUE DAWLEY RATS

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This study is designed to investigate the toxic effects of TiO₂-NPs in Sprague Dawley Rats. Total 25 rats weighted about 200 to 220 grams were purchased from Animal house University of Agriculture, Faisalabad. Rats were transferred and acclimatized at animal house of Government College University Faisalabad. For experimental setup rats were randomly divided into five groups (5 rats in each group) nominated as G1, G2, G3, G4 and G5 treated with TiO₂-NPs @ 0.00 (Control), 0.00 (Saline), 80, 120 and 160 mg/kg body weight for alternate 28 days. Weight was assessed weekly. After 28 days blood was collected in EDTA coated tubes from orbital sinuses. Serum was separately collected for biochemical parameters. Weight was significantly decreased as compared to control and saline. Hematological parameters Hb, HCT, Rbc, MCHC, lymphocytes and monocytes were decreased ($P < 0.05$) significantly while MCV and Wbc were increased significantly. Biochemical parameters ALT, AST, ALP, Urea, Creatinine, LDH and total bilirubin were increased significantly. Genotoxic parameters Length head and Head DNA (%) were significantly decreased and length tail, length comet, tail DNA (%) and tail moment were significantly increased. Hence it is concluded that Anatase Titanium dioxide nano particles induced genotoxic and hemato-biochemical effects in Sprague Dawley Rats.

DETECTING BIOMAGNIFICATION OF NICKEL HEAVY METAL IN FOOD CHAIN OF INSECTS UNDER SEWAGE IRRIGATED ECOSYSTEM

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Vegetables are well thought-out essential for balanced diet since these provide vitamins, minerals, dietary fiber, and photochemicals. Industrialization and urbanization have resulted in accumulation of heavy metals in vegetables grown in peri-urban areas due to accumulation of heavy metals cultivated in the usage of sewage waste water. From Punjab, previous studies have reported accumulation of heavy metals in water, soil and vegetables but none of these studies have evaluated these heavy metals in different insects (i.e. insects pests, predators, pollinators) found in this vegetable agro-ecosystem. Therefore current study is planned to investigate the accumulation of Ni heavy metal from vegetables grown in peri-urban areas of Multan. Different vegetables fields were selected that using sewage water. From these fields, samples of insect pests and pollinators were collected along with the samples of water, plant, and soil. These samples were analyzed by using the atomic absorption in order to quantify the Nickel by following standards assigned procedure. It was found that heavy metals Nickel was present in samples of insects pests,

predators and pollinators up to varying extent. Future studies should further investigate the impact of heavy metals on beneficial insects i.e. predators and pollinators. Since these are playing a vital role in our ecosystem.

EFFECT OF SEWAGE WATER ON WATER HYACINTH (*EICHHORNIA CRASSIPES*) IN CANALS OF SOUTHERN PUNJAB, PAKISTAN

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Water hyacinth, an invasive alien weed present in flowing as well as static water bodies. It has been called as one of the worst obnoxious weeds around the globe. It has become greater challenge for irrigation canals and water channels of Indus Water Basin in Pakistan because of its fast growth. In the Punjab, sewerage water is added to rivers causing proliferation of this weed. To assess correlation of its growth with waters in rivers polluted with urban sewage, this study was conducted. For the purpose, 5 canals viz. Wali Muhammad, Shujabad, Mailsi, Taleri and Muzaffargarh canals. Out of above mentioned, first three are diverged from the rivers having more sewerage water while last two canals are out shoot from river where less sewerage water is drained. According to the data recorded, growth of *E. crassipes* is noted in Wali Muhammad Canal as greatest followed by Shujabad, Taleri, Mailsi and Muzaffargarh Canals, respectively. This study can provide baseline for managing this noxious invasive weed as well as to help in making policies to make our rivers clean from urban wastes.

MICRONUCLEAR ABNORMALITIES IN ERYTHROCYTES OF *CYPRINUS CARPIO* UNDER THE EXPOSURE OF METALS MIXTURE

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Variety of heavy metals present on earth that are known due to their detrimental and noxious effects on the life of human beings as well as on aquatic organisms. Very narrow efforts has been done in the past to scrutinize the genotoxicity of mixture of metals in fish, *Cyprinus carpio* (common carp). Objective of this work was to assess the dose dependent genotoxic potential of metal mixture i.e. Pb+Cd+Co to *C. carpio* by using the micronucleus assay at constant laboratory conditions. Genotoxic potential of metals mixture was determined in terms of nuclear abnormalities under sublethal concentrations exposure of mixture for the period one month. Fish were exposed to sublethal concentrations viz. 1/3rd, 1/4th, 1/5th, 1/6th and 1/7th of LC₅₀, separately, along with control. During one month of exposure period, fish blood were sampled on weekly basis and directly smeared on slides for micronucleus assay. Nuclear abnormalities (%) were scored in terms of bi-nucleated, dumbbell, blebbed, notched and D-shaped nuclei. Data were statistically analyzed and results expressed in the form of Means±SD. Sensitivity of *Cyprinus carpio* to induce micronuclear abnormalities in blood erythrocytes varied significantly (p<0.05) under the sublethal exposure of Pb+Cd+Co mixture. Dose based increase in the micronuclear frequency was observed in blood cells of fish as compared to control group. At 1/3rd sublethal concentration of Pb+Cd+Co mixture, fish showed higher frequency (%) of nuclear damage as compared to control group. However, a clear dose-based elevation in nuclear abnormalities occurrence in blood cells of fish was observed. Toxicity tests establish the water quality parameters for fish as these test gave toxicant concentration (LC₅₀) that cause fish mortality even in short time. Different metals present in the form of mixtures in our aquatic environment therefore, it was a dire need to evaluate the genotoxic potential of metals in the form of mixtures on fish.

PROTECTIVE EFFECT OF QUERCETIN AGAINST DICLOFENAC INDUCED HEPATOTOXICITY IN ALBINO MICE

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Diclofenac sodium is a non-steroidal anti-inflammatory drug commonly used worldwide for the treatment of inflammation and pain due to arthritis or other ailments in man and animals. Despite these benefits, there are concerns that this drug may have adverse effect on animal's liver. This work was designed to determine the hepatotoxic effect of diclofenac sodium and protective effect of quercetin against diclofenac induced liver toxicity. Thirty-six were randomly divided into four groups with 9 rats per group. The first group served as control group that was treated with standard diet. The second group was intoxicated through oral administration of diclofenac sodium (8mg/kg, body weight) for twenty-one alternate days. Third group was treated with quercetin (10mg/kg, body weight) and fourth group was treated with quercetin in combination with diclofenac sodium. Three mice from each group were dissected every week. Various biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP) and albumin (ALB) from liver were studied. Oral administration of diclofenac sodium produced a significant ($p < 0.05$) increase in the liver weight, level of plasma ALT, AST and ALP and decrease in level of total protein and albumin. Compared with mice treated with diclofenac sodium alone, simultaneous supplementation of quercetin significantly decreased plasma levels of liver injury indicators. This study demonstrates the hepatoprotective activity of quercetin against diclofenac sodium induced toxicity and thus scientifically supports the use of flavonoids for the treatment of liver toxicity induced by different drugs.

ASSOCIATION OF PERSISTENT ORGANIC POLLUTANTS WITH LIVER ENZYMES IN DIABETIC PATIENTS OF KARACHI POPULATION

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In the world the prevalence of type 2 diabetes and POPs are increasing through the alarming rate and becoming a cause of serious health problem for public in the 21st century. Even though several epidemiological researches explain the fundamental link of POPs with liver enzymes and type 2 diabetes. To determine the association of persistent organic pollutants with liver enzymes in diabetic patient of Karachi population. Polychlorinated biphenyl and organochlorine pesticides exposure was closely associated with elevated liver enzymes and these elevated liver enzymes are the risk factor of type 2 diabetes. Our mean values result reveals that elevated liver enzymes such as (GPT, ALP and GGT) was higher in older age group (41-47) except GOT than other such as 27-33 younger and 34-40 middle age groups. Correspondingly mean level of pesticides was highly increases in younger age groups than older age groups. Statistically one way variance analyses (ANOVA) on SPSS version 22 concluded that on average GPT and GGT enzymes are equal or significant with adjusting age groups while on average GOT and ALP were not statistically significant with age groups. A significant increase found in GPT liver enzyme than other liver enzymes. We also apply logistic model (binary logistic regression) to analyze the change in liver enzymes level in the presence of various covariates such as pesticides, age, SBP, DBP and BMI on SPSS software version 22 and estimates were discussed for each enzymes. When pesticides increase by 1 mg/kg then average extreme GPT, ALP and GGT value goes down by 0.533, 0.210 and 0.053 and odd ratio goes in favor of extreme enzymes as 41.3%, 18.9% and 10.2% while average extreme GOT enzyme goes to increase about 0.097 with increase pesticides and odd ratio not set in favor of extreme GOT enzyme change about 10.2% while keeping other variable as constant except

pesticides. Similarly when other variables set as constant except age when age increase by 1 year the average extreme change in enzymes GPT, ALP and GGT goes down to about 0.060, 0.001 and 0.069 and odd ratio 5.8%, 0.1% and 2.3% goes in favor of extreme GOT enzyme as GOT increase with age about 0.022 and odd ratio is 2.3%. This analysis shows quantitative confirmation that support the exposure of polychlorinated biphenyls and organochlorine pollutants are closely associated with greater risk of elevation of liver enzymes and incidence of T2DM. These liver enzymes might be strong predictors of type 2 diabetes in the Karachi population. Other covariates such as age, blood pressure and body mass index also associate with change in liver enzymes. Conveniently collected samples indicate significant exposure of pesticides bioaccumulation in both men and women. At last we conclude from the present investigation that level of OCs and PCBs from biological parameter indicate that there is common occurrence of chemical bioaccumulation and bio magnifications of POPs in Karachi city.

EFFECT OF SALINITY ON GROWTH RATE AND SURVIVAL OF FISH

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Aquaculture aims to provide healthy and ideal environment to enhance fish growth and survival in a shorter time period. It is a great step to reduce hunger and poverty. Fish is an organism who bear salinity up to large range, including environmental parameters salinity is the most important which can affect rate of growth in fish. Euryhaline fish can bear the higher salinity level approximately $2\times$ seawater (60 g kg^{-1}). Some of the fish species tolerate salinity level up to 130 g kg^{-1} and they may be able to do this by switching their adaptive strategy when the level of salinity exceeds 60 g kg^{-1} . Fish can grow better at salinity level of almost 24‰. Salinity has a pronounced effect on fish growth at higher salinity level e.g. 4000 ppm. Different salinity levels were shown different effect on fish body functioning e.g., breeding, survival rate, food intake and osmoregulation. The rate of survival in fish also noted 100% at salinity level of 0 to 6‰. It is concluded that salinity has significant effect on fish growth and survival rate. Overall, salinity has higher impact on body functioning of fish.

ORGAN TOXICITY OF CHROMIUM VI TO ALBINO MICE AND AMELIORATIVE EFFECT OF *ALLIUM SATIVUM*

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Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) induced nephrotoxicity associated with oxidative stress. Garlic (*Allium sativum*) and its preparations have been widely recognized for the prevention and treatment of various diseases like cardiovascular and other metabolic diseases, atherosclerosis, and hyperlipidemia. Chromium VI is considered as a toxic transition heavy metal that causes severe damage to a variety of tissues and organs including the reproductive organs. The current study was designed to investigate the toxic effect of chromium VI on kidney and liver organs of albino mice and the ameliorative effect of garlic on Swiss albino mice. Mice were divided into four groups. Each group contained 4 male and 4 female albino mice treated with 0.2, 0.3, 0.5 and 1 mg/g body weight concentrations of potassium dichromate. The first group was given distilled water as a control. While the second group was given orally potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) 0.2 mg/g body weight as a dose group and the third group was given orally garlic extract as a 1.5 mg/g body weight as an antidote. The fourth group was administered with Potassium dichromate + garlic extract ($1.5+0.5\text{ mg/kg}$ body weight) as a dose + antidote. The higher dose of potassium dichromate caused severe toxicities in organs of albino mice. While comparison between organ weight and body weight of albino mice indicated that the value of organ weights for both liver and kidney is significant $P\geq 0.05$ while the value of body weight is non-

significant $P \leq 0.05$. *Histopathological examination of the liver of dose group showed mild vacuolar degeneration in cytoplasm of hepatocytes and kupffer cell hyperplasia*, Mild focal infiltration of inflammatory cells was also present. While Dose + Antidote group showed Perivascular infiltration and degeneration of cells. Antidote group showed mild infection. *Histopathological examination of the kidney of control group a normal appearance while dose group show mild degeneration of tubular epithelial cells and multiple foci of mononuclear cell in cortex*. Dose + antidote group show few of nuclei of tubular epithelial cell pyknotic and mild proteinous material in tubules. Antidote group showed deposition of inflammatory cells and detachment of tubular epithelial cells basement membrane. The results of this study demonstrate that extract of *Allium sativum* possess ameliorative effect on mice against chromium VI induced toxic effect in mice.

SERUM BIOCHEMISTRY OF CATLA CATLA AS INFLUENCED BY ZN AND MN TOXICITY

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A most important toxicity effect in aquatic system is due to the existence of heavy metals. This experiment was planned to analyze the variations in serum biochemistry of *Catla catla* affected by zinc and manganese toxicity. In the phenomena, fish seed hatchery was selected for the collection of fish fingerlings. Then they were shifted into the cemented tanks for one week for the purpose of acclimatization. After that the fish fingerlings were exposed to the metal mixture stressed condition in Zn+Mn toxicity for experimental trial. Controlled group of fishes were also kept in stress free aquarium for comparison. When the experimental trial was finished, samples of blood were collected from both treated and untreated groups. On daily basis a variety of water parameters were also recorded through the entire experiments trial. The results of serum biochemical parameters in the fish *Catla catla* are total protein amount was 2.1g/L, albumin 2.8g/L, cholesterol 5.10mmol/L, triglycerides 0.09mmol/L, ALT 770U/L, AST 705U/L, ALP 390U/L, sodium 144mmol/L, potassium 12.3mmol/L, chlorine 101mmol/L, and calcium 10.6mmol/L under the control of Zn+Mn toxicity. The findings of the results showed a considerable decline in all parameters except for four parameters that is AST, ALT, K⁺ and Cl⁻ that was elevated when exposed to the toxicity of metal mixture in the fish *Catla catla*. Considerable differences were observed between untreated and treated stressed fish *Catla catla* in statistical analysis. The inferences of conducted study were useful in understanding the function of serum biochemistry as a symbol of metal toxicity.

GENOTOXIC POTENTIAL OF DIETARY ENDOSULFAN ON CIRRHINA MRIGALA

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A diverse array of agricultural and industrial chemicals containing pesticides and other xenobiotics are contaminating the aquatic environments in which pesticides are biologically non-degradable that can cause toxicity in animals through oxidative damage to membrane lipids, DNA and proteins. This study reveals dietary endosulfan induced genotoxic damage in peripheral blood erythrocytes of 150-day old fish, *Cirrhina mrigala* using Comet assay under controlled laboratory conditions. Fish were exposed to four different sub-lethal concentrations viz. 10%, 20%, 33% and 50% LD₅₀ of dietary endosulfan, for 30 days along with positive (Cyclophosphamide) and negative control groups, separately. Peripheral blood of chronically exposed fish was examined for percentage of damaged nuclei (%), genetic damage index (GDI) and cumulative tail lengths of comets (μm). Chronic exposure of dietary endosulfan to *Cirrhina mrigala* induced higher DNA damage in peripheral erythrocytes of fish that varied significantly ($p < 0.05$) with exposure concentrations along with control groups. The 50% LD₅₀ exposure of dietary endosulfan caused significantly higher percentage of DNA damage, GDI and cumulative tail length to the comets induced in the nuclei

while it remained significantly lower due to negative control. This study reveals that Comet assay can be used as useful tool for the determination of genotoxic effects of pesticides on fish.

CHRONIC EXPOSURE EFFECTS OF CADMIUM AND CHROMIUM ON THE GROWTH PERFORMANCE OF FRESHWATER CARNIVOROUS FISH, *WALLAGO ATTU*

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The rivers in Pakistan are becoming highly polluted with metals as a result of increasing anthropogenic activities leading to various disturbances in the health and well beings of carnivorous fish fauna. Therefore, the present research work was conducted to determine the effects of sub-lethal (1/3rd of LC₅₀) concentrations of heavy metals viz. cadmium and chromium on the growth responses of fish, *Wallago attu*. The three length groups (50, 100 and 150 mm) of *W. attu* were stressed with metals, separately while control groups remained un-stressed. The growth performance of fish was determined in terms of increase or decrease in average wet weights (g) and total lengths (mm), feed intake (g), feed conversion efficiency (%) and condition factor. Fish were subjected to sub-lethal cadmium and chromium concentrations, separately, under controlled laboratory conditions for 120 days and their growth was compared with that of the control fish. The data on all the defined growth variables were collected on weekly basis and statistically analyzed by using the Factorial design (RCBD) and Tukey's/Student Newman-Keul tests. The results revealed that cadmium and chromium stress to the fish caused significant reductions in the growth performance of all the three length groups of fish as compared to the control. After sixteen weeks, all the three length groups viz. 50, 100 and 150 mm stressed with cadmium and chromium attained significantly lower weights as 0.14±0.05 and 0.13±0.05 g, respectively, as compared to the control fish. The total length increments of three length groups of treated and control fish exhibited statistically significant differences. The average total length increments of fish followed the order: 150 mm > 100 mm > 50 mm. All the un-stressed (control) fish species showed significantly (p<0.05) lower condition factor values as compared to the treated fish species. However, among the three length groups, 50 mm fish exhibited significantly better condition factor than that of 100 and 150 mm fish length groups. There existed statistically significant differences for feed intake of treated and control fish groups. The control fish showed significantly higher feed intake as compared to the treated fish. The differences among three length groups for feed intake varied significantly also. Highly significant differences were recorded for feed conversion efficiencies of cadmium and chromium stressed three length groups of fish that followed the order: 50 mm > 100 mm > 150 mm.

CADMIUM CONCENTRATION IN WATER AND *LABEO ROHITA* FISH COLLECTED FROM LOCAL RIVER RAVI, DISTT LAHORE

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Heavy metals are a big source of water pollution and cause serious health problems for Aquatic organisms by assembling in their tissue. From fish these enters in the food chain and incidentally it is a risk for consumers. Present study was conducted to investigate concentration of Heavy metal in locally consumed fish species *Labeo rohita* sold at local fish market in Distt Lahore. The concentration of heavy metal Cadmium (Cd) C was measured in their gills, liver, muscle and intestine by atomic absorption spectrophotometry by wet digestion of sample. The water sample from the same ponds was also collected and analyze for the metal concentration for above said Heavy metals. Results indicated that the level Cadmium (Cd) was significantly lowest in fish muscle (1.15±0.13) as compared to liver (1.70±0.03, intestine (1.63±0.11) and gills (2.22±0.17). Water of river Ravi was found highly contaminated with Cadmium concentration (2.22±0.21). The estimated daily intakes (EDI) of all metals (lg/day/person) through consumption of the fish species by Palestinian people in the Gaza Strip were well below the permissible tolerable

daily intake for 70 kg person (PTDI70) set by FAO/WHO. Therefore, it can be concluded that no problems on human health would be raised at present from the consumption of commercial fish from the River Ravi fish sold in local markets. However, precautionary measures must be taken to stop further accumulation.

ASSESSMENT OF HEAVY METALS IN HERBIVORE FISHES OF RIVER CHENAB

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Fish is economically very important. It is not only a source of food but also valuable in terms of trade. The aim of the study was to explore the quality of fish present in River Chenab through some efficient and convenient technique that is Atomic Absorption Spectrometry. The present study evaluated the accumulation level of Cadmium, Chromium, Copper and Lead in two herbivorous fish species (*Labeo calbasu* and *Cirrhinus mrigala*) captured from River Chenab. Significant higher value of Cadmium accumulation (ppm) was observed in *Labeo calbasu* (0.596 ± 0.27) followed by *Cirrhinus mrigala* (0.212 ± 0.04). No significant difference regarding chromium (Cr), copper (Cu) and Lead (Pb) accumulation was observed in *Labeo calbasu* (0.627 ± 0.38 , 0.345 ± 0.13 , 1.000 ± 1.70) and *Cirrhinus mrigala* (0.487 ± 0.06 , 0.255 ± 0.05 , 0.912 ± 0.28) respectively. Our study clearly revealed that River Chenab to some extent is a polluted river of Pakistan.

GROWTH RESPONSES OF CARNIVOROUS FISH UNDER CHRONIC EXPOSURE OF METALS MIXTURE

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The research work was conducted to determine the effect of sub-lethal ($1/3^{\text{rd}}$ of LC_{50}) concentrations of heavy metals mixture (Cd+Cr+Co+Cu+Ni) on the growth performance of two carnivorous fish species viz. *Channa marulius* and *Mystus seenghala*, under controlled laboratory conditions. The three length groups (50, 100 and 150 mm) of each fish species were divided into two groups i.e. treated and control. For a period of 120 days, each group of fish species was exposed to Cd+Cr+Co+Cu+Ni mixture, separately and growth of the treated fish was compared with that of the control fish group. The growth performance of three length groups of metals mixture treated and control fish groups was monitored in terms of increase/decrease in average wet weights (g) and total lengths (mm), feed intake (g), condition factor and feed conversion ratio. The data on all the growth variables of fish were collected, on weekly basis and analyzed statistically. Results showed that all the three (50, 100 and 150 mm) length groups of Cd+Cr+Co+Cu+Ni mixture treated fish revealed significantly reduced growth, in terms of increase in average wet weights, total lengths and feed intake as compared to the three length groups of control fish. However, the treated fish showed significantly higher values of condition factor (K) and feed conversion ratio (FCR) as compared to the control fish. Regarding overall means, the 150 mm fish showed significantly better average wet weight and total length increments, feed intake and FCR as compared to 100 and 50 mm length groups while 50 mm fish exhibited significantly better K value than that of 100 and 150 mm length groups of fish. During chronic Cd+Cr+Co+Cu+Ni mixture exposure, *C. marulius* exhibited significantly ($p < 0.01$) higher average wet weight and total length increments, feed intake, K and FCR as compared to *M. seenghala*.

COMPARISON OF HEAVY METALS (CADMIUM, CHROMIUM, COPPER, LEAD AND ZINC) CONCENTRATION IN FARMED FISH *LABEO ROHITA*, *CATLA CATLA*, AND *CIRRHINUS MRIGALA*

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To measure the heavy metals concentration in farmed fish. Three carp species were sampled for three different local fish farms of Bhone, district Jhang. Sample of fish were randomly collected from sampling station. Five heavy metals, Cd (cadmium), Cr (Chromium), Cu (Copper), Pb (Lead) and Zn (Zinc) were analysed in four organs liver, muscle, intestine and gills of three carp species. Zn was found significantly high in all five metals and gills show maximum concentration of metals because of their direct contact with water followed by liver, intestine and muscle. Highest concentration of Zn was found in the gills of *Catla catla* (44.70 ± 4.13) and the lowest concentration were found in the muscle of *Labeo rohita* (7.07 ± 0.55). All the metals were found above the permissible limits set by FAO. This study proven that the fish cultivated in the farms of Bhone, district Jhang are not safe for human use it create severe health problems for consumers use these farmed fish.

ASSESSMENT OF HEAVY METALS IN CARNIVORE FISHES OF RIVER CHENAB

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Fish is economically very important. It is not only a source of food but also valuable in terms of trade. The aim of the study was to explore the quality of fish present in River Chenab through some efficient and convenient technique that is Atomic Absorption Spectrometry. The present study evaluated the accumulation level of Cadmium, Chromium, Copper and Lead in two carnivorous fish species (*Wallago attu* and *Sperata sarwari*) captured from River Chenab. Significant higher value of Cadmium accumulation (ppm) was observed in *Sperata sarwari* (0.577 ± 0.21) followed by *Wallago attu* (0.355 ± 0.06). No significant difference of chromium (Cr), copper (Cu) and Lead (Pb) was observed in *Wallago attu* (0.260 ± 0.04 , 0.565 ± 0.31 , 1.375 ± 1.33) as well as in *Sperata sarwari* (0.242 ± 0.03 , 0.225 ± 0.13 , 0.212 ± 0.06) respectively. Our study clearly revealed that River Chenab to some extent is a polluted river of Pakistan.

IMPACT OF LEAD ON FRUIT FLIES IN MULTAN, PUNJAB PAKISTAN

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The heavy metals like lead are important toxic and dangerous material which directly or indirectly contaminate the foods, soil water, air, accumulate in insect body like fruit fly, causes organogenesis, sperm abnormalities, damage to nervous system, morphogenesis syndromes and miscarriage. The current study was carried out to check the lead effect- ion on various developmental stages of fruit fly at Muhammad Nawaz Shareef University of Agriculture, Multan in 2018. For this purpose, sex pairs of flies were collected from field and added to culture consist different concentrations of lead-ion for mating and egg lying. During the present study, the transformation rate of larva-pupa, pupa-adult and adult-egg lying, morphological changes and time required for each stage were studied. The different morphological changes like elongated wings, de-shaped wings, elongated and folded legs were observed in current study. The study resulted that larval and pupation period was increased with 25-300 mg/L culture- medium lead- ion, while alteration of larvae to pupa and pupa to adult reduced. The lead has negative effect on egg hatching period. The

larval, pupal and adult growth rate was reduced with metal concentration. The study concluded that lead interfere with enzymes or hormones that evolved in metamorphosis and ATP synthesis.

EVALUATION OF THE HEAVY METALS CONCENTRATIONS; LEAD AND CADMIUM IN DIFFERENT ORGANS OF MAHSEER (*TOR PUTITORA*) OF INDUS RIVER AT BATAKRA, GHAZI AND KUND, DISTRICT SWABI

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Heavy metals are considered as the most dangerous water contaminants because it accumulate in fish body and cause toxicity. This study tried to observed bioaccumulation of heavy metals such as lead and cadmium in gills, swim bladder and muscles of *Tor putitora* collected from three different locations of River Indus that were Batakra, Ghazi and Kund in district Swabi, Khyber Pakhtunkhwa. Present study was conducted from February to May 2016. Fish sample were collected from Batakra in February, from Ghazi in March and in April from Kund. Our study revealed that estimated accumulation of heavy metals in fish sample collected from Batakra in February was in order of Pb (0.03) > Cd (0.2) in gills and muscle tissues Pb (0.02) > Cd (N.D) of *Tor putitora*, while no cadmium has been observed in swim bladder. Similarly, fish sample collected from Ghazi in March having heavy metals concentration in order of Pb (0.20) > Cd (0.01) in gills as well as Pb (0.02) > Cd (N.D) in swim bladder. While in muscle order of observed heavy metal concentration was Cd (0.02) > Pb (0.01). Likewise, in fish sample collected in April from Kund having heavy metals concentration in order of Pb (0.30) > Cd (0.22) in gills while in swim bladder Cd (0.01) > Pb (N.D). While in muscle tissues both lead and cadmium was not detected. The results of our present study revealed that heavy metals were present in River Indus but its quantity was in normal range and have no adverse effects on fish growth, survival and reproduction and that's the reason we found maximum fish diversity of fish in River Indus in district Swabi.

EVALUATION OF THE HEAVY METALS CONCENTRATIONS; LEAD AND CADMIUM IN DIFFERENT ORGANS OF COMMON CORP (*CYPRINUS CARPIO*) OF INDUS RIVER AT HUND, TOPI AND KUND, DISTRICT SWABI

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Heavy metals are considered as the most dangerous water contaminants because of its possibility of bioaccumulation in fish organs and its toxic effects on fish as well as on human being via food chain. In this regard the present study was conducted to investigate the accumulation of heavy metals such as lead and cadmium in gills, swim bladder and muscle tissues of *Cyprinus carpio* collected from three different locations Hund, Kund and Topi of River Indus at Swabi in month of February, March and April, 2016 respectively. Heavy metals entered in fish bodies by three possible ways that are by gills, body surface and digestive track, and after entering, it accumulate in different organs of fish, which are toxic to fish, as well as transfer to human being via food chain so that's why the human being effect as well. Our study revealed that, accumulation of heavy metals in month of February in gills, swim bladder and muscle tissues were in order of Pb (0.06) > Cd (0.04) and Cd (0.05) > Pb (N.D) and Pb (0.05) > Cd (N.D) respectively. Likewise, the present study unveiled the accumulation of heavy metals in month of March, in organs gills, swim bladder and muscle tissues were in order of Pb (0.20) > Cd (0.01) and Pb (0.11) > Cd (0) and Cd (0.02) > Pb(N.D) respectively. The estimated accumulation of heavy metals of Common carp collected in April month were in order of Pb (0.20) > Cd (0.02) in gills tissues while in swim bladder and muscle tissues Pb and Cd has not been observed. Overall heavy metals concentration was maximum in month of March (Kund) followed by February (Topi) and April (Hund). The results of our present study revealed that heavy metals are present in River

Indus but its concentration lies below the toxic level thus do not affect the fish. Therefore, if pollution problems are controlled in present area of District Swabi so it will provide more favorable conditions in Indus River for fish growth, survival and reproduction, otherwise if pollution is incremented with such ratio so very soon, the aquatic fauna as well as human being via food chain can be affected.

SCRUTINIZING BIOACCUMULATION AND HISTOPATHOLOGY OF KIDNEY AND INTESTINE OF GRASS CARP (*CTENOPHARYGODON IDELLA*) AFTER EXPOSURE TO WATER BORNE TOXICITY LC 50 OF HEAVY METALS; COPPER, LEAD AND CHROMIUM

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Heavy metals are considered as the most dangerous water contaminants because of its possibility of bioaccumulation in fish different organs it has toxic effects on fish as well as human because they consume fish in their diet. Heavy metals enter the fish body through possible three ways which includes gills, body surface and digestive track. After entering to body through these possible ways it accumulates in different organs and has adverse effects on fishes as well as humans when they consumed it in the form of diet. In this regards present study was conducted to find out bioaccumulation of heavy metals in intestine and kidneys of grass carp when feed on LC₅₀ for 24, 42, 72 and 96 hours respectively. The results of our study revealed that those groups which are feed with LC₅₀ for 24 hours it has been observed in them that bioaccumulation of heavy metals were more in intestine than kidney tissues. From our work it has been observed that lead was accumulates more than other two heavy metals which are chromium and copper. More over the results of our work also revealed that lead accumulates more in kidney tissues but their level are lower than its accumulation in intestine tissues. The results of our study revealed that those fishes which are feed with LC₅₀ for 48 hours shows that accumulation of heavy metals were more in intestine as compared to intestine tissues. From our study it has also been observed that lead accumulates in more level those other two heavy metals. Those fishes which have been feed with LC₅₀ for 72 hours shows that accumulation of heavy metals were considerably high in intestine tissues than kidney tissues. Also lead accumulation was more than other two metals. In our conducted study variation in results were present in those fishes which are feed with LC₅₀ for 96 hours. Accumulation of heavy metals was more in kidneys tissues as compared to intestines. In the prospect of our study it is strictly recommended to government to take action to control environmental pollution to provide best environment to aquatic life especially fishes which are the main diet of human all over the world.

BIOACCUMULATION OF HEAVY METALS IN THE GILLS AND MUSCLES TISSUES OF *PUNTIUS SOPHORE* IN RIVER SWAT

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The present study was shown to determine the concentration of heavy metals such as Zn, Pb, Cr and Ni, in muscles and gills of economically importance fish species *puntius sophore* in river swat at swat, Khyber Pakhtunkhwa, Pakistan. The fish samples (*puntius sophore*) were collected in June, July and August 2016 in river swat at swat. In the *puntius sophore* concentration of heavy Metals zinc (Zn) distribution pattern was in the increasing order in the muscle were landaki ≥ fizaghat ≥ Odigram, the gills were Odigram ≥ fizaghat ≥ landaki. Pb distribution order in the muscles and gills were fizaghat ≥ landaki ≥ Odigram. The chromium distribution order in the muscles were landaki ≥ fizaghat ≥ Odigram, in gills were Odigram ≥ landaki ≥ fizaghat. Nickel distribution order in the muscles were fizaghat ≥ landaki ≥ Odigram, gills were fizaghat ≥ landaki ≥ Odigram. Significant difference the p value is less than 0.05 (P < 0.05) between the tissues. The mean concentration of metals like Zn, Pb, Cr and Ni in

Fizaghat (station 1), muscle were 0.27 ± 0.17 , 1.0 ± 0.1 , 1.70 ± 0.30 , and 1.10 ± 0.23 ppm/g respectively, in gills were 0.63 ± 0.03 , 1.2 ± 0.4 , 1.10 ± 1.00 and 0.93 ± 0.13 ppm/g respectively. Odigram (station 2) in muscle tissue the mean concentration were 0.19 ± 0.05 , 0.71 ± 0.14 , 1.43 ± 0.20 , 1.10 ± 0.23 ppm/g respectively, in gills were 0.65 ± 0.03 , 0.34 ± 0.09 , 1.78 ± 0.12 , 0.23 ± 0.003 ppm/g respectively. The concentration of heavy metals at landaki (station 3) in muscle tissue were 0.35 ± 0.15 , 0.92 ± 0.12 , 3.055 ± 0.29 and 0.82 ± 0.29 ppm/g respectively. In gills were 0.24 ± 0.03 , 0.37 ± 0.12 , 1.28 ± 0.43 and 0.75 ± 0.22 ppm/g respectively. In *puntius sophore* mean concentration of heavy metals all the selected site in the muscle at Odigram recorded the lowest concentration of Zn (0.19 ± 0.05). Muscles at landaki recorded highest concentration of Cr (3.055 ± 0.29) the mean concentrations of heavy metals were in the acceptable limit providing by different food and water agencies (FAO, 1983).

EFFECTS OF CADMIUM EXPOSURE ON STRUCTURAL AND FUNCTIONAL ALTERATIONS OF RENAL TISSUE: SETTING *IN VIVO* MODEL IN RATS

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Cadmium is a heavy metal that has received considerable concern environmentally and occupationally. It has a long biological half-life mainly due to its low rate of excretion from the body and is known to be a widespread environmental contaminant and a potential toxin that may adversely affect human health. Kidney is a main organ affected by cadmium exposure and has gained the most attention in recent years. Current study aimed to assess the toxic effect of cadmium exposure on haematological parameters and alteration in kidney structure for setting an *in vivo* model. Nine male Wistar rats were randomly assigned to the following groups: control, low-dose CdCl₂ (2 mg/kg body weight) and high-dose CdCl₂ (4 mg/kg body weight). The rats continuously received CdCl₂ via food for 8 weeks. Blood samples were collected at day 30 and 45 for haematology. Rats were sacrificed at the end of experiment and kidneys were removed for histology. The results indicated that various haematological parameters Hb, HCT, MCH, MCV, WBC, PLTs, RDW, MONO, GRAN and LYM were decreased in cadmium administrated rats. RBC count showed no changes at 30 days while eventually decreased in number at 45 days. The histopathological changes were mainly – the glomerular swelling (at low dose), the shrinkage of glomerulus (at high dose), increased spaces between bowman's capsule and glomerulus, reduced cell density, depletion in proximal and distal tubular area, lesions were caused due to degeneration of glomerulus and renal tubules. This animal model may be used in further research for various purposes.

BIO-ACCUMULATION OF DIFFERENT HEAVY METALS IN LIVER & LUNGS OF *UROMASTYX HARDWICKII*

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Anthropogenic and natural activities are the major sources of Bio-accumulation and bio transfer of heavy metals. The environmental contamination due to heavy metals has been considered as the major issue due to its deleterious effects upon biotic community. In the present study *Uromastyx hardwickii* was captured and used as a bio indicator of heavy metals in the natural habitat of lizards. 150 samples of *Uromastyx hardwickii* were captured from the desert (south region) of Mianwali, Bhawalnagar, Cholistan, Thar and Rajanpur by using capturing kits and Iron cage, transferred to lab safely. 15 samples irrespective to their genders were selected, tagged (U-01 to U-15) and weighed. Maximum weight of female *Uromastyx hardwickii* was 309.5g while that of male *Uromastyx hardwickii* was 2306.6g. Samples were dissected and organs were preserved in 30% formalin. Then wet digestion of the organs was performed which gives a clear solution of the organs which later used for analysis of heavy metal (Cr, Ni, Pb, Cu and Cd) by FASP. Results indicated that concentration of Cr, Ni, Cu, Cd and Pb in liver was in the range of 0.00-4.00 µg/g, 4.00-7.50 µg/g, 5.00-10.50 µg/g, 0.00-0.50 µg/g and 1.50-15.50 µg/g respectively. Similarly concentration

of Cr, Ni, Cu, Cd and Pb in lung was found in the range of 0.00-3.00 µg/g, 4.50-8.00 µg/g, 4.00-19.50 µg/g, 0.00-0.050 µg/g and 4.00-25.00 µg/g respectively. Inter matrices co relation coefficient values was obtained for different concentration of heavy metals in lung and liver of the samples which showed that liver of *Uromastix* would be more relevant in accessing different heavy metals. Bioaccumulation of heavy metals in liver may be due to major role of liver in body metabolism and detoxification of food absorbed from the small intestine. Results of this study proved that *Uromastix hardwickii* can be used as a reliable diagnostic tool for the estimation of different heavy metal within the natural habitat of lizards. However there is need to collect more data in future studies from different socio cultural areas so as to be able to determine possible relationship and differences peculiar to each area in terms of heavy metal distribution in soil and different body parts of most suitable indicator animal species within the vicinity.

ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN TWO FISH SPECIES *CIRRHINUS MRIGALA*(MORI) AND *LABEO ROHITA*(ROHU) COLLECTED FROM CHANGHOZ DAM DISTRICT KARAK, KP, PAKISTAN

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Pollution of water bodies by heavy metals has become a worldwide problem in the last decades. Heavy metals enter to the aquatic ecosystems through different natural and human sources, such as industrial and domestic sewage, use of pesticides and inorganic fertilizers in agricultural practices, runoff from the catchment area during rainy seasons, geomorphological weathering of the earth's crust and atmospheric deposition. This study was conducted from to assess heavy metals (cadmium, zinc, iron, copper, nickel and lead) concentration in fishes, water and soil of changhoz dam district Karak, K.P Pakistan. For this purpose two species of fishes namely *Cirrhinus mrigala* (mori) and *Labeo rohita* (rohu) from different sides and four samples of water and four samples of soil from different regions of changhoz dam were collected during the month of February and March 2015. For acid digestion 2g of each dried and grinded samples was weighed correctly and then transferred to 50ml conical flask. Then 10mL concentrated HNO₃ (70%) and 2mL H₂O₂ catalyst was added to the flask. After 30min the flask was placed on hot plate. Gradually the temperature of heating block was increased, starting from 50°C up to 160°C. The digestion of each sample was completed in 6-8 hours with the repeated addition of concentrated HNO₃ and H₂O₂ catalyst. The appearance of approximately 5ml water-like colorless solution was the sign of digestion completion. After the completion of digestion, the solution was placed to make it cool. Then the solution was filtered by Whatman filter paper # 42 into 50ml self-standing plastic bottles with the plugs tightly sealed. By adding 2ml concentrated nitric acid (70%) the volume of the solution was raised to 7ml. Then the solution was diluted by adding 25ml distilled water. Then the bottle was labeled and used for metal analysis. Estimation of elements of interest was carried out by Atomic Absorption Spectrometer. During analysis the calibration standard for each element were prepared from standard stock solution and analyzed at regular time intervals as to check the flow of the instrument. In *Cirrhinus mrigala* (Mori) highest concentration of Pb was observed in scale while Cu was not detected in abdomen, Ni was not detected in tail and Cd were not detected in any part, Fe and Zn were detected concentrations below the permissible limit. In *Labeo rohita* (Rohu) highest concentration showed by Ni in abdomen while in other part Ni along with Cd were not detected; Fe and Pb were observed within the permissible limit. While Zn and Cu were found below the permissible rang. In water of four sides highest concentration was showed by Ni in front side Zn and Cd showed less concentration while Fe, Cu and Pb were detected within the permissible limit. In soil highest concentration was showed by Fe while Ni was not detected in any sample. Pb showed normal concentration; Zn, Cd and Cu were found in less concentration than the normal permissible rang. From the above study it may be concluded that in *Cirrhinus mrigala* (mori) highest level of pb was detected while in *Labeo rohita* (Rohu) highest concentration showed by Ni in abdomen and remaining all the analyzed metals were below the permissible limit.

ASSESSMENT OF HEAVY METALS AND THEIR EFFECTS ON THE LIPIDS PROFILE OF THE SELECTED FISH SPECIES (*HYPOPHthalmichthys MOLITRIX* AND *CATLA CATLA*) OF TANDA DAM, KOHAT

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This study was conducted to assess heavy metals (iron, nickel, copper, zinc, cadmium, Chromium and lead) concentration in fishes, water and sediment of Tanda dam district Kohat, K.P. Pakistan. For this purpose, two species of fishes namely *Hypophthalmichthys molitrix* and *Catla catla*, water and sediment from three different sides of Tanda dam were collected from January to March 2016. For acid digestion 2g of each dried and grinded samples was weighed correctly and then transferred to 50ml conical flask. Then 10mL concentrated HNO₃ (70%) and 2mL H₂O₂ catalyst was added to the flask. After 30min the flask was placed on hot plate. Gradually the temperature of heating block was increased, starting from 50°C up to 160°C. The digestion of each sample was completed in 6-8 hours with the repeated addition of concentrated HNO₃ and H₂O₂ catalyst. The appearance of approximately 5ml water-like colorless solution was the sign of digestion completion. After the completion of digestion, the solution was placed to make it cool. Then the solution was filtered by Whatman filter paper # 42 into 50ml self-standing plastic bottles with the plugs tightly sealed. By adding 2ml concentrated nitric acid (70%) the volume of the solution was raised to 7ml. Then the solution was diluted by adding 25ml distilled water. Then the bottle was labeled and used for metal analysis. Estimation of elements of interest was carried out by Atomic Absorption Spectrometer. During analysis the calibration standard for each element were prepared from standard stock solution and analyzed at regular time intervals as to check the flow of the instrument. In *Hypophthalmichthys molitrix* highest concentration (22.5mg/kg) of Pb was observed in the scales, while Cd has the lowest concentration (0.016mg/kg) in the scales amongst all metals. Pb, Fe and Ni were observed above the permissible limit by FAO and WHO. In *Catla catla* highest concentration showed by Pb in the Tail (32.9mg/kg), while Cd have the lowest concentration (0.016mg/kg) in the abdomen. The concentrations of Pb, Fe and Ni were observed above the permissible limit by FAO and WHO, in *Catla catla*. In water samples the highest concentration (15.40-19.802mg/L) was showed by Pb and Cd showed the lowest concentration (0.16-0.12mg/L). In case of sediment Pb (12.5 mg/kg), Fe (5.435mg/kg), Cu (5.568mg/kg) and Cd (0.16mg/kg) were found above the normal range. From the above study it may be concluded that accumulation of heavy metals decreases the lipid quantity of fish. The concentration of heavy metals in *Catla catla* was higher than *Hypophthalmichthys molitrix*. While the lipid level in *Catla catla* was less than *Hypophthalmichthys molitrix*.

ACCUMULATION PATTERNS OF NICKEL IN THE BODY ORGANS OF CARNIVOROUS FISH *MYSTUS SEENGHALA* AT ACUTE CONCENTRATIONS EXPOSURE

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The contamination of riverine systems of Pakistan with the increasing metallic ions concentration has resulted into serious consequences towards health and survival of the indigenous carnivorous fish fauna of economic value. Therefore, the research work was planned to determine 96 h acute toxicity (LC₅₀ and lethal concentrations) of commonly found contaminant i.e. nickel to the carnivorous fish *Mystus seenghala* of 150 mm total length. The toxicity trials were performed under constant laboratory conditions. The fish mortality data were recorded and analyzed statistically through Probit analysis method with 95% confidence interval limits to determine 96 h LC₅₀ and lethal concentrations of nickel for *M. seenghala*. At 96 h LC₅₀ and lethal concentrations exposure, metal accumulation in skin, gills and gut of the fish was also analyzed by using atomic absorption spectrophotometer. The 96 h LC₅₀ and lethal concentration values of nickel computed for *M. seenghala* were 85.32±2.49 and 117.19±5.26 mg

L⁻¹, respectively. Statistically highly significant differences at $p < 0.01$ were recorded among the selected body organs of fish to accumulate nickel. At both 96 h LC₅₀ and lethal concentrations exposure, nickel amassing in body organs of *M. seenghala* followed the order: gills > gut > skin.

BIOACCUMULATION AND TISSUES ALTERATION IN GRASS CARP AGAINST HEAVY METALS: AN EXPERIMENTAL APPROACH

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Health and environmental problems arising from heavy metals present in the aquatic ecosystem and their bioaccumulation and tissues alteration in fishes are very well known. The present study was conducted for the purpose to explore the toxicological effects of LC₁₅ of heavy metals copper, lead and chromium on gills and muscles tissues of grass carp (*Ctenopharyngodon idella*). Fish were exposed for 24, 48, 72 and 96 hrs respectively and gills tissues accumulated maximum concentration of lead (4.11, 1.036, 1.646 and 3.72 mgL⁻¹) followed by chromium (0.5, 0.048, 0.47 and 0.736 mgL⁻¹) and copper (0.26, 0.03, 0.02 and 0.036 mgL⁻¹). Muscles tissues also absorbed maximum concentration of lead (6.69, 3.14, 3.253 and 3.73 mgL⁻¹) followed by chromium (0.59, 0.51, 0.47 and 0.307 mgL⁻¹) and copper (0.03, 0.033, 0.02 and 0.013 mgL⁻¹) respectively. In addition gills and muscles tissues were also observed for different histological changes inter lamellar space, mild lamellar fusion, irregular cells, cellular necrosis in the secondary lamellae, hyperplasia, bleeding of tissues, cartilaginous hyperplasia of gill rays, inflammatory cells, destruction of epithelial cells were observed in gills tissues while in muscles tissues different histological alterations like; swelling and necrosis of muscle fibers, degeneration of muscle fibers, edema of muscle bundles, inflammation, zig zag of muscle fibers and lesions in muscle tissues were noticed in fish exposed with different doses of these heavy metals indicating the toxicity of metals to aquatic fauna as well as to human being via food chain.

ANALYSIS OF TOXICOLOGICAL EFFECTS OF HEAVY METALS; COPPER, LEAD AND CHROMIUM ON HEMATOLOGICAL INDICES AND ERYTHROCYTES CELL AND NUCLEUS MORPHOLOGY OF GRASS CARP (*CTENOPHARYGODON IDELLA*)

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Heavy metals accumulated in the tissues of aquatic organisms and cause toxicity when the accumulation reaches to high level. The lethal effects of pollutants are determined by the most common method that is hematological techniques. Thus, in the current study blood parameters such as Hb, RBC, monocytes and lymphocytes were observed under the toxicological effects of heavy metals copper, lead and chromium for 24, 48, 72 and 96 hours. In addition, the erythrocytes cell and nucleus morphology were also under the observation in the present study. Pb, Cr and Cu toxicant affect caused alteration in blood parameter of grass carp. Decline in concentration of Hb was observed against all heavy metals and maximum decline was observed against chromium at 96hrs exposure. Similarly, RBC concentration also decline against each metal and maximum decline was noticed against chromium after exposure of fish for 96hrs. Likewise monocytes concentration also decreases against each metal and maximum decrease in concentration was noticed against chromium at 72hrs exposure while inclined in concentration was also observed in lymphocytes and maximum inclined was noticed against chromium when fish was exposed of 24 and 72 hrs respectively. Thus, LC₅₀ of chromium heavy metals showed maximum toxicity to blood parameters of grass carp followed by copper and lead. In addition, red blood cells and nucleus morphology was also observed and abnormalities like enlargement of cell, irregular cell membrane and ruptured cell membrane were observed in cell

while abnormalities like micro nucleus, lobed nucleus and irregular nucleus were observed in nucleus. Therefore, the present study explores the toxicological effects of each heavy metal indicating that these heavy metals can alter the ecosystem of aquatic organisms.

AGE AND SPECIES SPECIFIC SENSITIVITY OF COPPER SULFATE TO FRESHWATER CYPRINIDS

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For ecological risk assessment, investigating the effects of pollutants on all life stages of an organism is prerequisite. Here, sensitivity of four age groups viz fertilized eggs to larvae, swim up fry, advance fry and Fingerling of Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*) to water borne copper sulfate was investigated. An experiment was conducted in glass aquaria under control condition and repeated three times. Each group was exposed to different concentration of CuSO₄ and mortality per treatment was noted after 24, 48, 72 and 96 h. Median lethal concentration (LC50/96h) of copper sulfate to each group of both species was determined by probit analysis. Results indicated age and species specific response. Among all groups, fertilized eggs of both species appeared more sensitive towards copper toxicity. The mean sensitivity of both fish species, as their LC50 96 h followed the order: fertilized eggs > swim up fry > advance fry > Fingerling. Moreover, *L. rohita* appeared more sensitive in contrast to *C. mrigala*. This study provides baseline information for toleration of the maximum quantity of copper by different developmental stages of the both species and suggested that LC50 of heavy metals depends not only on species but also on the developmental stages of a specific organism under test.

HEPATOPROTECTIVE EFFECT OF WITH ANIASOMNIFERA ON ARSENIC INDUCED TOXICITY IN RABBITS

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The present study is done to investigate the effect of arsenic toxicity in rabbits and to evaluate the hepatoprotective effects of the ayurvedic plant *Withaniasomnifera* root powder (WSRP) in toxicities rabbits and compare the toxic effects with the reference antioxidant ascorbic acid. Investigations comprising of 1 month period, 36 Rabbits were divided into 6 groups. WSRP was administrated orally (100mg/kg of body weight) in rabbits to group 2 and 3, Ascorbic acid (200mg/kg of body weight) to group 4 and 5 along with arsenic in group 3, 5 and 6 (10mg/kg of body weight) while 1st was control group. Body weights, liver weights and serum biochemical profile were compared with control and toxin group of all treated groups. The data were compared statistically by using mini tab. Results indicate that *W. somnifera* plays an important role in decreasing the level of SGPT, Alkaline phosphatase and AST in blood to cope with arsenic toxicity. Moreover, it also decreases the liver weight that increases due to arsenic toxicity. Thus, *Withaniasomnifera* can be used as a hepatoprotective against arsenic induced liver damage.

STUDY OF HUMAN BLOOD LEAD LEVELS IN RELATION TO LEAD IN STREET DUST DRINKING WATER AND DEMOGRAPHIC CHARACTERISTICS SUBJECTS OF CITY AND ADJOINING AREAS OF HYDERABAD PAKISTAN

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Objective to study the human blood levels in relation to lead in street dust drinking water and demographic characteristics of city and adjoining areas of Hyderabad Pakistan. Methodology. Cross sectional study of blood lead levels, lead in dust and drinking water was undertaken in Hyderabad city and its adjoining areas. The dust and water samples were analyzed for lead content. 480 adult subjects from city and 522 subjects from adjoining areas were also analyzed for blood lead. A questionnaire was also used to determine the association of blood lead and demographic characteristics. Results. The mean lead content of the dust samples in city, and adjoining areas was found as $255 \pm 12.6 \mu\text{g/g}$ and $320 \pm 15.2 \mu\text{g/g}$ respectively. The mean lead content of municipal water in city and adjoining areas was found as $40.16 \pm 4.3 \mu\text{g/l}$ and $35.72 \pm 6.4 \mu\text{g/l}$ respectively. The ground water was also found highly contaminated with lead in both of the areas and findings showed that none of the water samples met WHO recommended safe value of $10 \mu\text{g/l}$ of lead in drinking water. The mean blood lead levels of subjects from city and adjoining areas was found as $13.72 \pm 5.83 \mu\text{g/dl}$ and $20.97 \pm 9.37 \mu\text{g/dl}$ respectively having positive association with certain demographic characteristics of subjects. Conclusion. Decaying garbage stagnant water in residential areas, weathering of domestic paints and battery recycling are main reasons for the elevated lead concentration of lead in the environmental media and human subjects of Hyderabad city and its adjoining areas.

ANALYSIS OF MALATHION ENVIRONMENTAL INTOXICATION IN MAMMALIAN TESTICULAR STRUCTURE AND FUNCTIONS: A HISTOLOGICAL ASSESSMENT STUDY

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In recent times, the use of organophosphate insecticides improved human life standard and yield better results in agriculture but its demerits and detrimental effects on humans and other wildlife systems are a great problem of everybody's concern. Technical grade of Malathion, used in the current study, is one of the most commonly used insecticide in agriculture crops, in stored grain houses, homes, gardens, and for outdoor purposes have been recorded several reproductive effects in mammals so far. In current study 24 adult male albino mice, weighed between 25-30 grams were used. Animals were treated orally with 20mg/kg/day, 50mg/kg/day and 100mg/kg/day of Fyfanon (EC-57% w/v), a local commercial product of Malathion, to study the testicular structure and spermatogenesis. After 20 days of treatment, animals were dissected for histological examinations. The one way analysis of variance (ANOVA) showed a significant ($p < 0.001$) increase in seminiferous tubule epithelial height whereas, the seminiferous tubule lumen diameter was significantly ($p < 0.001$) reduced in animals treated with 50mg/kg/day and 100mg/kg/day when compared with sham. The number of round spermatids is significantly ($p < 0.01$) and ($p < 0.001$) reduced in 50mg/kg/day and 100mg/kg/day treated animals, respectively. There is no significant ($p > 0.05$) change in animals treated with a dose of 20mg/kg/day. Possibly, this increase of epithelial height and decrease in lumen diameter is perhaps because of the implication of more accumulation of vascular fluid from interstitial compartment and swelling of cells. However, the results concluded that Malathion has somewhat severe degenerative effects in mammalian reproductive systems.

BIOTOXICITY OF LOCAL ISOLATES OF *BACILLUS THURINGIENSIS* FOR THE CONTROL OF MOSQUITO

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The present study was aimed to control resistant insects by isolating soil samples from different localities intended for effective mosquitocidal *cry2*, *cry4*, *cry9*, *cry11* positive *Bacillus thuringiensis* (*B.t.*). Control of various mosquito species at various developmental larval stages is a useful measure to mitigate transmission of pathogens by mosquito vectors. Various species of *B.thuringiensis* reported to be toxic against mosquitoes, black flies. Hundred Strains have been isolated from different localities of Punjab from diverse habitats, including soil, insects, stored-product dust, and deciduous and coniferous leaves. 15 *B.t.* strains were screened, 75% were from dry, leaf liter, garden soil samples, 16% from animal waste and 10 % from moist soil of crop area. Genomic DNA was isolated and a DNA fragment of 650bp, 439bp of *cry11*, *cry4* gene was amplified by PCR respectively. Seven *Bacillus thuringiensis* were brought to be positive for *cry11* and *cry4* gene. The 16S rDNA study exposed that these screened *B.t* confirmed 99% homology with *B.t.* serovar *tolworthi*, *B.t.* str. *Al Hakam*, *B.t.* serovar *thuringiensis*, *B.t.* serovar *konkukian*, and *B.t.* serovar *Chinensis*. *B.t.* serovar *Indiana*, *B.t.* serovar *kurstuki*. The toxicity bioassays with *B.t.* spores proved that six *B.t.* isolates harboring *cry11* and *cry4* genes (viz., NF1*B.t.*, 2,3,4,5,6,7) were most toxic to 3rd instar larvae of mosquito, *Aedes aegypti*, *Anopheles stephensi*. Among six *B.t.* isolates, GCU-DAB-NF5 was found the most toxic and was isolated from moist soil containing a Dirty sewage water LC₅₀ is 522.027±0.17 µg /ml against *Aedes aegypti* (third instar larvae) and showed 100% mortality at 1000µg of spores/ml. The positive control HD-500 showed 94% mortality. It was found that LC₅₀ (522 µg/ml) of GCU-DAB-NF5 is quite less than HD500 LC₅₀ (673µg/ml). So, GCU-DAB-NF5 is more toxic as compare to HD500. Among these *Bt* 2 shows the same LC₅₀ higher than HD500 against *Anopheles stephensi* harbouring *cry11* and *cry4* gene. All isolates did not show the same level of toxicity which reflects the variation in expression level of *cry11*, *cry4* gene present in local *B.t.* isolates. The study provides a convenient method which is time saving and economical. This study recommends that *B. thuringiensis* at spore stage provides good mortality percentage.

ASSESSMENT OF HISTOPATHOLOGY AND GENOTOXICITY IN FISH EXPOSED TO PLASTICIZERS, DI-METHYL PHTHALATE AND DI-N-OCTYL PHTHALATE

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Healthy juveniles of *Ctenopharyngodon idella* were injected intramuscularly with phthalates, Di-methyl-phthalate, (DMP), Di-n-octyl Phthalate (D-n-oP) and mixture of both Phthalates for 3, 6 and 15 days, respectively. Histo-pathological alterations in gills were examined in juveniles of *C. idella* of varied weight (61.00±11.44 g) and length (8.40±0.37). Analysis of condition factor (C.F.) for fish health assessment revealed that C.F values of 6- and 15-days phthalate exposures in both treatment groups showed significant lower mean values as compared to the 3-days exposed group indicating poor meat quality and toxic impact of phthalates. Comparison of total protein content in all the treatment groups showed statistically significantly highest fluctuations in the protein contents. Hematological analysis of *C. idella* revealed significant decrease in Total RBCs count, Hemoglobin concentration, Hematocrit and platelet count in all treatment groups at p<0.05. Significant histological alterations in gills were epithelial lifting, fusion of primary and secondary gill lamellae, epitheliocystis, expansion of basal epithelial cells of gill arch and hyperplasia. HAI_{Gills} for phthalate injected fish followed the order: HAI_{15-day} > HAI_{6-day} > HAI_{3-day}.

STUDY ON RELATION OF PARAKEET, *PSITTACULA KRAMERI* TO THE PATHOGENIC FUNGUS, *ALTERNARIA SOLANI*

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Tomato, *Solanum lycopersicum* (family, *Solanaceae* L.) is one of the most important vegetables used in homes and as processed food. Tomato fruit is attacked by many pests and plant pathogens. Rose ringed parakeet, *Psittacula krameri* is one of the most destructive bird pest of orchids and horticultural crops. It damage the fruits by gnawing and wounding which is exposed to many pathogens. Early blight is the most damaging disease of tomato caused by the fungus *Alternaria solani*. After parakeet invasion the wounded tomatoes are exposed more to this fungi which spread rapidly to all over the plant causing enormous damage. In this regard a study was carried out at 15 fields in Bund Murad. Damage estimation of tomatoes by parakeets was carried out. The average percentage of tomatoes damaged per field computed was 1.02%. Damaged and diseased samples (n=25) were brought to the laboratory to isolate the pathogen, infected portions of fruit samples were surface sterilized (70% ethanol and 0.1% mercuric chloride) and cultured on Potato Dextrose Agar (PDA). Plates were incubated for seven days at 25 °C. Cultured fungus was identified based on morphological characters of spores and mycelium. Out of 25 samples 14.75 samples were found positive for the fungus *Alternaria solani*.

HORMONAL ALTERATIONS IN MALE NEW ZEALAND RABBITS (*ORYCTOLAGUS CUNICULUS*) EXPOSED TO MALATHION AND CYPERMETHRIN

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Insecticides exposure poses a serious risk to animals, environment and the public health. The widespread use of these insecticides in agriculture, veterinary and public health has acted as a stimulus for the study of their toxicity to non-target organisms, so the purpose of this study is to evaluate the toxic effects of Malathion and Cypermethrin on hormonal indices including thyroid and sex hormones. Nine male rabbits (*Oryctolagus cuniculus*) were intoxicated through oral intoxication of Malathion and Cypermethrin at a dose of 75mg/kg body weight for consecutive seven days. Rabbits were grouped into three groups A, B and C comprised of three animals each. Group A was considered as the control, Group B was the Malathion fed group while C was the Cypermethrin intoxicated group. Both insecticides were fed through 1cc syringe. Blood samples were collected and preserved in a refrigerator for further analysis. Demonstrated the toxic influences of both insecticides. Malathion produced an increase of 8.08% in LH and 17.13% in FSH, a decrease of 8.03% in estrogen, 45.59% in progesterone, 32.34% in T3, 2.06% in T4 and 31.72% in testosterone. Cypermethrin caused an increase of 12.72% in LH and 51.82% in FSH, a decrease of 13.39% in estrogen, 7.86% in progesterone, 39.65% in T3, 5.02% in T4 and 39.44% in testosterone. This study reveals that both the insecticide is toxic by causing alterations in the hormonal indices and caused reproductive as well as thyroid abnormalities.

ASSESSMENT STUDY OF BIOMAGNIFICATION OF ARSENIC AND CHROMIUM IN SNAILS OF GENUS *PHYSA* (GASTROPODA: MOLLUSCA) IN FAISALABAD

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The present study aims to assess ecological risk of two heavy metals (arsenic, chromium) to fresh water snails belonging to genus *Physa* in Faisalabad. The snails are very important part of the food chain and are excellent source

of calcium for the birds during their breeding season. The snails of genus *Physa* were sampled during the months of October 2017 to March 2018 from the selected 62 villages of Faisalabad and were identified using SSR markers. The foot, shell, water and soil samples were digested and tested for the presence of the heavy metals using atomic absorption. The data was subjected to a suitable statistical analysis. The three species *Physa fontinalis*, *Physa acuta* and *Physa gyrina* were found. The results showed the chromium concentration in the selected villages lies in the range between 0.063mg/l to 4.445mg/l. The arsenic concentration was found to be in the range of 0.00104mg/l to 0.15818mg/l. The results obtained were found to be higher than the standard NEQ's i.e 0.01mg/l for arsenic and 1.0mg/l for chromium. The study will provide the status, diversity and presence of heavy metals in the study area. This study will be a baseline information to identify water-quality-related ecological threats in the Faisalabad region.

BIO-ACCUMULATIVE ABILITY OF EARTHWORMS FOR SELECTED HEAVY METALS

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Earthworms are most common and diverse invertebrate group on earth. They play significant role in decomposition as well as fertility of soil. The aim of this study was to evaluate the bio accumulative ability of earthworms for Zinc and Chromium. For this purpose, samples were collected from the cropland area of University of Agriculture, Faisalabad, Punjab, Pakistan. Then, specimens were acclimatized for 48 hours. In a randomized block design, treatments of Zinc sulphate and Chromium sulphate were given at 200 and 300 mg/kg soil. The total time period of one month was taken for this experiment, in which, the temperature, humidity and pH were maintained. After 30 days, earthworm's weight was measured and morphological parameters were resolved and contrasted at first recorded parameters. Biomass was also recorded prior to the experiment and then compared with the biomass after 4 weeks. A gradual decrease in biomass was observed in metal treated worms. While, increase in weight was seen in controlled group. Length of worms were ascertained and contrasted with introductory length following 30 days exposure period, when presented to various treatments. A steady reduction was seen in earthworms *Pheretima* species of night crawlers. The processing of earthworms was done in controlled and treated environments with Cr₂SO₄ and ZnSO₄. To check the efficacy of earthworms toward the heavy metals with the help of spectrophotometer. All results were observed via ANOVA. Accumulation of Zinc and Chromium were seen in all the specimens. It was resulted that earthworms have more ability to absorb the zinc in their bodies.

8. VIROLOGY

SEROPREVALENCE OF CYTOMEGALOVIRUS IN PREGNANT WOMEN OF DISTRICT KARAK, KP, PAKISTAN

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The chief objective of the study is to determine the population-based seroprevalence in pregnant women and to further explore the prevalence and risk factors associated with Cytomegalovirus infection among the pregnant women in the district Karak Khyber Pakhtunkhwa. 150 blood samples were collected from pregnant women by attending different healthcare center of district Karak. Samples were analyzed through ICT tests for Cytomegalovirus-specific IgG antibodies. Total of 150 samples 44(29.33%) were positive. CMV seroprevalence increased gradually with age, and were high in age group 34-39 year (37.50%). Analysis of CMV infection among pregnant women were based on their education, the Cytomegalovirus infection prevalence was very high in Illiterate (32.81%) as compared to graduated women. Similarly, prevalence of CMV infection among pregnant women in housewife was (32.94%) and was high as compared to employed women. Similarly, the prevalence of Cytomegalovirus infection based on different areas is variable. It is higher in rural area than urban area. The Cytomegalovirus infection has a very high prevalence and taken an endemic form in Pakistan, so routine screening of CMV among pregnant women is recommended.

EPIDEMIOLOGICAL STUDY OF FOOT AND MOUTH DISEASE IN LIVESTOCK OF DISTRICT LAKKI MARWAT, KHYBER PAKHTUNKHWA, PAKISTAN

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Foot-and-mouth disease (FMD) is an infectious viral disease of cloven-footed animals. It is widespread in Pakistan with huge economic losses. The chief objective of the study was to estimate the sero-prevalence of FMD in livestock in district Lakki Marwat, southern part of Khyber Pakhtunkhwa (KPK) Pakistan. A total of 200 sera were collected from buffalo and goat during December 2017 to May 2018. The sera were examined using Immuno chromatographic technique (ICT). The current study indicated that overall FMD in Livestock were 14.31%. Among these FMD in small ruminants were 16.37% while in large ruminants were 12%. A significant difference in sero-conversion was observed in small, young and adult of both small and large ruminants. The sero-conversion was highest in adult animals (20.33% in small ruminants and 13.63% in large ruminants) followed by young animals (15.45% small ruminants and 12.5% large) and kids (14.03% small ruminants and 8.69% large ruminants). FMD viral activity both in small and large ruminants were high in the month of December (20% small ruminants and 16% large ruminants) followed by January, February, March, April and May. The Seroprevalence of FMD was significantly lower in both small and large ruminants having good health status (16.35% in small and 10% in large ruminants) while high in those having weak health status (17.91% in small and 15.7% in large ruminants). The higher sero-prevalence of disease has substantial economic implications which signify the need for devising effective control measures.

SEROPREVALENCE OF MEASLES IGG ANTIBODY IN CHILDREN IN DISTRICT BANNU

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Rubella is an infectious viral disease causing rash and fever in normal, however when contracted by pregnant women cross placenta and directs to infection of a developing fetus causing Fetal death, abortion, premature birth or Congenital Rubella Syndrome. 200 Blood samples were collected pregnant women randomly in different health centers of both urban and rural area in District Lakki Marwat, in a span of five months from December 2014 to March 2015. Sera samples were screened for rubella IgG and IgM antibodies by ELISA. Our findings revealed that 32 (16%) respondents were positive for IgG and 5(2.5%) for IgM of the total. Higher sero-positivity rates were found in the age group of 33-38 years. Our result also indicate that majority of participants were positive in 2nd trimester for both IgG(18.82%) and IgM (3.52%). Women residing in rural area show high sero-positivity rate (IgG=18.8% and IgM=2.56%) than urban (IgG=12.04 and IgM=2.4%). This study acknowledged prevalence rate of rubella virus infection among pregnant women and find out magnitude of this disease in southern parts of Khyber Pakhtunkhwa Pakistan which is the former study to the best of our knowledge.

STIMULATOR OF INTERFERON GENES (STING) OF MANDARIN FISH (*SINIPERCA CHUATSI*) MEDIATES ANTIVIRAL SIGNALLING THROUGH INDUCTION OF TYPE I INTERFERONS

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Stimulator of interferon genes (STING) is an essential transmembrane protein which plays a key role as a central mediator of the cytosolic DNA sensors (CDSs) and retinoic acid-inducible gene I (RIG-I) like receptor (RLR) signalling pathway which activates type I interferons (IFNs) in innate antiviral response. In the present study, a STING homolog from mandarin fish (*Siniperca chuatsi*) (ScSTING) was cloned and characterized to understand its potential roles in innate immunity. Sequence analysis showed that ScSTING is encoded by a peptide of 417 amino acids which comprises five transmembrane domains at its N terminus and a STING superfamily domain. The gene expression analysis showed that ScSTING is constitutively expressed in all selected organs/tissues and with the stimulation of poly I:C, a significant increase in mRNA transcripts was observed. Furthermore, significant induction in the ScSTING mRNA expression was observed in spleen and head-kidney tissues as well as in mandarin fish fry (MFF-1) cells post infectious spleen and kidney necrosis virus (ISKNV) infection. However, a delayed (72 h) up-regulation in the transcription of ScSTING was observed which indicated a virus mediate inhibition of the gene expression during early phase of infection. Meanwhile, poly I:C stimulation results in the rapid and robust (6h) up-regulation in the mRNA expression. Moreover, over-expression of ScSTING alone or along with TBK1 in MFF-1 cells leads to the significant induction in the expression of type I IFNs and ISGs (Mx, ISG15 and VIPERIN). Furthermore, ScSTING over-expressed cells showed significant reduction in the ISKNV titers. Taken together, these results indicate that ScSTING is involved in the innate antiviral immune responses through TBK1-IRF3/IRF7-IFN/ISGs pathway, using both CDSs and RLR signalling pathway.

DEVELOPMENT OF ELISA FOR NEWCASTLE DISEASE VIRUS (NDV)**Zahra Naz* and Moazur Rahman**¹*National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan*²*School of Biological Sciences, University of the Punjab, Lahore, Pakistan*

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Non-glycosylated matrix (M) protein is one of the abundant structural protein of Newcastle disease virus (NDV) which is the causative agent of Newcastle disease (ND). Newcastle disease is a common infection of many birds including poultry and poses a constant threat to the agriculture and livestock of a country. Considering the conservancy and role of M protein in the viral life cycle, it can be targeted to diagnose NDV. The present study was designed to validate the importance of M protein for developing an indirect enzyme linked immunosorbent assay (ELISA) based study to detect Newcastle Disease in poultry. The recombinant M protein was expressed in *Escherichia coli* and was then purified in its soluble form using the zwitterionic detergent lauryldimethylamine oxide (LDAO). Multiple serum samples were collected from different poultry farms of district Faisalabad. The soluble M protein was then used as the coating antigen in the indirect ELISA for NDV antibodies detection in the samples. The results confirmed the potential role of M protein for NDV detection. This study provides the basis to establish a matrix protein-based indirect ELISA for detection and quantification of the anti-NDV antibody titer in the serum of affected chicken. In order to analyze a large number of samples, the proposed ELISA can be optimized and up-scaled for rapid and reliable detection of NDV infection.

INFLUENZA; AN EMERGING DISEASE IN THE COMMUNITY**Shafia Saba^{1,2}, Ata-ur-Rehman Khan², Unsar Naeem-Ullah^{1*}, Muhammad Ramzan¹**¹*Department of Entomology, Muhammad Nawaz Shareef University of Agriculture, Multan*²*Epidemic Prevention and Control Programme, District Health Authority, Multan*

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Influenza (Seasonal) commonly known as Flu is a contagious respiratory illness caused by Influenza Viruses (A, B, C, D). Influenza A Viruses can be categorized as Avian influenza or Bird flu (H5N1 & H9N2), Swine influenza or Swine Flu (H1N1 & H3N2), or other types of animal influenza viruses. Pakistan is a South Asian country with tropical to temperate climate, and influenza is observed to demonstrate a divergent pattern in temperate areas with a distinctive climax in winter usually in December- April. Pakistan is facing regular pandemics of influenza in different parts of the country since the first case was reported in 2009. The current study is based on the patients records collected during the flu season 2016 from Nishtar Hospital Multan, Punjab Pakistan. Total of 75 patients were reported with the symptoms of Influenza from Jan-Mar 2016 out of which 29 were positive for Influenza A (H1N1), while the rest were infected by some other viruses. All the patients were reported with fever from 2-10 days, cough and shortness of breath (SOB). The male female ratio was 37:38, within the age groups of 8 months to 75 years. Seven mortalities were also occurred, 5 of them were females and the two were males. A female toddler (7 months) was also expired. Rest of the patients were recovered and discharged from hospital. It is need of the time to sensitise the public for necessary preventive measures against the flu and arrangement of the vaccines at government level before the start of flu season.

9. ANATOMY

CRANIOMETRICAL ANALYSIS OF WILD BOAR (*SUS SCROFA*) FROM NORTHERN PUNJAB, PAKISTAN

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The current study reports the cranial morphology of *Sus scrofa*, the most widely spread representative of suidae family, with detailed comparison of different features of skull from northern Punjab, Pakistan. Cranial morphology is used to discriminate between the different species and sub species of the family suidae and for differentiation between the wild and domesticated suids. The studied material comprises the three skulls. The straighter snouts and slenderer crania manifests that specimens under study were wild while the sex of species was determined by the permanent canine teeth morphology, as in females the upper canines extend in ventrolateral directions and continue to grow in lateral direction while in males the upper canines extend out in anterolateral direction and curve dorsally. The age of suids was determined on the basis of eruption of third molar. Among studied specimens the individuals belong to different age groups hence for further comparative analysis the only one specimen which is observed as adult was studied. The 51 craniometric and 20 mandibular measurements were carried on the adult male skull and data derived from analysis of our sample was compared with previously reported data of European wild boar in case of craniometrical features while mandibular values were compared with Japanese wild boar. As a result of comparison of the different craniometric and mandibular parameters it was demonstrated that the studied specimen has larger dimensions for various parameters than the European and Japanese wild boar. The smaller cranial and mandibular values may be due to some evolutionary and climatic changes or hunting pressure.

SECTION – I I

PESTS AND PEST CONTROL

WEIGHABLE LOSSES AND PHYSICAL DESTRUCTION CAUSED TO TRITICUM AESTIVUM BY KHAPRA BEETLE (*TROGODERMA GRANARIUM* EVERTS) INFESTATION

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Khapra beetle *Trogoderma granarium*, Everts. Is one of the most destructive and quarantine stored product pests throughout the world. During the present study effect of khapra beetle were seen on three wheat varieties (Abadgar, TD-1 and Moomal). Material of this beetle was collected from the different localities (Sakrand) whereas the fresh samples of wheat varieties were collected from the wheat research institute, Sakrand town. During the observation three parameter weight loss, insect damaged grain and insect undamaged grain of percentage were seen in the three months April, May, June-2018. During the three month experimentation of *Trogoderma granarium*, it was noticed that Max: weight loss (16.9 ± 8.66) and Insect damaged grain (34.02 ± 7.28) were recorded on wheat variety Abadgar during the June-2018. Their, Min: weight loss (10.2 ± 6.13) and Insect damaged grain (24 ± 4.98) were recorded on wheat variety Moomal during the April-2018. In contrast to these parameters, Max: Insect undamaged grain (29.7 ± 6.37) was recorded on wheat variety Moomal during the April-2018. Whereas, Min: was recorded (24.7 ± 2.05) on wheat variety TD1 during the June-2018. During the investigation large amount of weighable losses and physical destruction in three wheat varieties were seen. This is the first time study of khapra beetle and wheat varieties from the Wheat research institute, Sakrand, Sindh, Pakistan.

IMPROVING APPEALING TENDENCY OF METHYL EUGENOL BAIT
IMPACT OF SELECTIVE INSECTICIDES AND ABIOTIC FACTORS ON PREDATORY
FAUNA IN *Bt* COTTON FIELD PLOTS

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Injudicious use of synthetic insecticides for the control of insect pests of cotton negatively impacts large number of biocontrol agents including some effective chrysopidae species and spiders. Great number of these ecofriendly biocontrol agents do exist in cotton fields. Current study was carried out to observe the efficacy of selective insecticides against different bio-control agents in two *Bt* cotton cultivars. Two varieties of *Bt* cotton (single *Bt* 142 and double *Bt* CEMB 33) were used to evaluate predators associated with *Bt* cotton. The efficacy of some selective insecticides viz: acephate (Commando plus[®]), neotinoid (Oshin[®]), bifenthrin (Talstar[®]), spintoram (Radiant[®]), spirotetramat (Movento[®]) and nitenpyram (Maximal[®]) was evaluated against green lacewing and predatory spiders. In general a significant difference was found for all applications when compared with untreated plots. The range of 0.18% to 0.58% population of *Chrysoperla carnea* and 0.18% to 0.64% predatory spiders was observed in single *Bt* before insecticide application. While after insecticide application the results were different as the population of both *C. carnea* and predatory spiders was reduced to 0.11% and 0.12% respectively. Similarly, in double *Bt* 0.21% to 0.55% population of *C. carnea* and 0.41% to 0.74% population of spiders was observed before insecticide application, while after insecticide application, population of *C. carnea* was reduced to 0.10% and predatory spider's population to 0.12%. Overall results demonstrated that the maximum population of bio-control agents was observed after treated with Radiant[®] and Oshin[®]. While very less numbers of predators were observed after using Commando plus[®], Movento[®] and Maximal[®]. There was 0.11% population of *C. carnea* in both varieties showed by Commando plus[®] and 0.12% population of spider in both varieties showed by Movento[®] and Maximal[®]. Correlation matrix of

bio-control agents and abiotic factors showed positive correlation of *C. carnea* with abiotic factors (temperature and humidity) while spider exhibited negative correlation with these abiotic factors. It is thus concluded that insecticides have hazardous effects on bio-control agents but some insecticides, such as spintoram (Radiant®) and neotectinoid (Oshin®), may be safer for them. However, judicious and proper use of recommended chemistries should also be implicated. Furthermore, insecticidal control of cotton insect pests during different seasons may also affect spider's population especially and other predators generally.

EVALUATION OF DIFFERENT CORN GLUCOSE BASED BAITS AGAINST HOUSE FLY IN LABORATORY AND FIELD STUDIES

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Musca domestica, L. (Diptera: Muscidae), House fly is a sanitary pest which is very important and spread infectious diseases in livestock and also in humans; safer replacement of the insecticides and their formulations is needed. The baits were prepared with different types of glucose syrup, powder milk, neonicotinoid insecticides (Imidacloprid and Thiacloprid) and flower extracts (Marigold and Sunflower). Adult flies were collected by hand netting from different dairy and poultry farms and placed into rearing cages for mass production. Varying proportion of glucose syrup with fixed milk powder and insecticides/flower extracts was arranged in Completely Randomized Design (CRD) with five replications. Jar test was developed to determine the bait efficacy. Data of bait efficacy was collected after 6, 12, 24 and 48hrs exposure of flies to bait and was analyzed under tukey method. Results obtained revealed that Imidacloprid was proved to be highly toxic to house fly in the present experiment by causing 82.2% mortality after 48 hours. The mortality increased with the passage of time. Thiacloprid caused 66.2% mortality after 48 hours. In flower extracts Marigold with 30% concentration 80% was proved to be highly toxic after 48 hours. Sunflower (30% concentration) was less toxic 78.2% than Marigold (30% concentration) after 48 hours. These baits of flower extracts and insecticides with corn glucose can be used to minimize the effect of house flies which cause annoyance and irritation to people, animal, poultry and livestock units, respectively.

STUDIES ON FORMULATION AND FIELD EVALUATION OF BAIT CONTAINING *Cordia myxa* WOOD EXTRACTS AGAINST SUBTERRANEAN TERMITES

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Termites are social insects and they are found all over the world. Termites cause damages to wooden products. Wooden products can be prevented by the natural defensive chemicals present in wood extractives. In the present studies, wood extractives of *Cordia myxa* were elaborated for their antitermitic activities. For this purpose wood of *Cordia myxa* was dried and shaving was prepared. Three solvents methanol, petroleum ether and n-hexane were added in to shavings and bottles were made airtight by placing aluminum foil at lid. These bottles were kept away from sunlight in dark. Contents of the bottles were shaken at intervals of 3 days and mixture was kept for 30 days. Three concentrations (5mg mL⁻¹, 10mg mL⁻¹ and 15mg mL⁻¹) were made from the crude extracts obtained. Laboratory experiment was conducted in CRD Complete randomized design with three replications. In the laboratory filter paper was treated with each concentration of *C. myxa* wood extractives and placed in petri dishes. To check the mortality a known number of 50 termites were released in each petri dish. Mortality was observed at 24 48 72 and 96 hours after exposure. Maximum mortality of (97.50 and 92.50%) was observed for 15mg mL⁻¹ and 10mg mL⁻¹ concentration of methanol respectively at 96hours post-exposure time interval. Efficacy of baits prepared from *C. myxa* wood extractives and maize cob powder were tested in laboratory. For this purpose baits were placed in petri dishes and 50 termites were released in the petri dishes. Mortality was observed at 24 48 72 and 96hours of exposure. Among treatment maximum mortality values (100 and 95%) were observed in case of baits treated with 15mg mL⁻¹ and 10mg

ml⁻¹ n-hexane concentrations. Field experiment was conducted to check the efficacy of baits in the field. In field experiment baits of (18 ×18 ×18mm) were treated with all extracts. For control treatment baits were treated with solvents only. Maximum attack of termites (90%) was seen in control treatment. Minimum mean termite individual (0.25 at 7 days to 17.75 at 28 days) was recorded in baits treated with 10mg ml⁻¹ and 5 mg ml⁻¹ concentrations of methanol.

EVALUATION OF NATURAL RESISTANCE AND ANTITERMITIC PROPERTIES OF *BAUHINIA VARIEGATA* (FABACEAE) WOOD EXTRACTS AGAINST SUBTERRANEAN TERMITES

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Termiticide activity of *B. variegata* wood extractives in four organic solvents i.e. n-hexane, petroleum ether, ethyl acetate and water via cold extraction at three different concentrations (10%, 20% and 30%) in respective solvents was studied. Deterrent and repellent activities were determined by using each concentration of extractives. Stakes of *Poplar deltoides* (10 cm×5 cm×2 cm) were oven dried at 80°C, 100°C and 150°C for 7 days. Finally wooden blocks of poplar (10 cm×5 cm×2 cm) were treated with extractives by dipping technique. Poplar stakes were also treated with six combinations of wood extractives in 1:1 ratio. These treated wooden blocks were exposed to termites in the field in accordance with Randomized Complete Block Design (RCBD). Data on weight loss of wooden blocks in each experiment was recorded after exposure to the termite at 60 days, and analyzed by ANOVA followed by post-hoc test. In the field experiments, choice and no-choice bioassay, minimum percent weight loss (0.93%) was observed in fresh *B. variegata* stakes while maximum (55.5 %) was observed in *P. deltoides*. In case of without oven dry method, after 36 and 72 hours maximum percent weight loss (53.65 and 55%) in heartwood extractives was observed in control treatment of n-hexane whereas minimum percent weight loss (3.80 and 2.61%) was seen in stakes treated with n-hexane at 30 mg/ml. Similarly, after 36 and 72 hours maximum percent weight loss (69 and 75.33%) in bark extractives was observed in control treatment of water whereas minimum percent weight loss (1.67 and 1.09%) was seen in stakes treated with n-hexane at 30 mg/ml. Results of efficacy of combination of heartwood and bark extractives showed maximum percent weight loss in case of water + hexane combination (22.1 and 22.11 %) after 36 hours. After 72 hours same combination gave maximum percent weight loss (70.6 and 64.8%). While, minimum percent weight loss in heartwood and bark extractives was shown by n-hexane + ethyl acetate combination after 36 hours (3.07 and 2.5 %) and 72 hours (4.48 and 3 %). In case of oven dry method, at 80°C, n-hexane heartwood extractives showed minimum percent weight loss (2.03 and 5.51 %) at 30 mg/ml after 36 and 72 hours. At 100°C, n-hexane extractives showed minimum percent weight loss (3 and 3.29 %) at 30 mg/ml after 36 and 72 hours. At 150°C, n-hexane extractives showed minimum percent weight loss (1.71 and 1.11 %) at 30 mg/ml after 36 and 72 hours. Likewise, at 30 mg/ml, bark's extractives in n-hexane solvent showed minimum percent weight loss after 36 hours (2.49 and 4.49 %) at 80 and 100°C. After 72 hours, at 30 mg/ml same solvent showed minimum percent weight loss (1.46 and 6%) at the same temperature. At 150°C, maximum percent weight loss (65.50%) was observed in control treatment of water while minimum (7.44 %) was seen in wooden stakes treated with n-hexane at 30 mg/ml.

STUDIES ON EXTENT OF PREVENTION FROM TERMITES BY APPLICATION OF HEARTWOOD EXTRACTIVES OF *ACACIA NILOTICA* ON POPULAR WOOD

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Heartwood of *Acacia nilotica* is very durable and resists termites and fungal attack. Extractives of *Acacia nilotica* can be an effective wood preservative for a non-durable wood such as poplar wood, which was selected for the study of its termiticidal nature. Two types of woods of *Acacia nilotica* were used for extraction, one separated from tree freshly and another cut from tree that was kept for few months in stores of timber Market. Extractives from

both the types of heartwoods were prepared by boiling their shreddings in hot water and evaporating for concentrated extracts. Three concentrations of both the types of extractives (10%, 20% and 30%) were made in water. Experiments were analyzed using Completely Randomized Design owing to uniformity of the field condition. Dipping method was applied to treat wooden chunks of poplar wood with each of concentration. After application and compensating hygroscopicity, chunks were exposed to the termites' infestation in concrete soil pits prepared as test arena. The weight loss at 30% concentration was minimum for fresh wood extractive (11.4%) and old wood extractives (5.7%) in contrast to control (untreated) group of both extractive types (75.7% and 56.4%). Application of extractives was done in experiment No.2 by the best concentration of extractives after reducing the moisture contents of poplar wood up to <50% and >50% by oven drying. The poplar wood was seasoned to reduce moisture contents less than 100%. Wooden stakes with <50% moisture contents was found with minimum weight loss% (9.55%) and for >50% weight loss (15.65%) was found. Wood treated with highest temperature 160°C was noted with minimum weight loss (31.3%) at 72hrs dipping interval with old wood extractive type. Wood treated with old wood extractives for 72hrs has weight loss (9.6%). Chunks were placed for termites' exposure after weighing. In experiment No. 3, poplar chunks were oven dried at varying temperatures and exposed to termites' attack after weighing. Treated woods were kept for considerable time period not less than one month and afterward chunks were carried to Termites Research Laboratory and were cleaned with brush to remove dirt and dust. Weight loss before and after the exposure to the termites will be determined on laboratory weight balance. For the effect of seasoning at varying temperature, weight loss at 160°C was (14.1%) for fresh and (13.8%) for old wood extractives Weight loss% was noted to be very high in control treatments of all experiments.

STUDIES ON EXTENT OF PREVENTION OF WOOD FROM TERMITES BY IMPREGNATION WITH CASTOR BEAN OIL

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Vegetable oil is one promising such material that can be utilized as a wood preservative. Castor bean oil was selected for the study of its termiticidal nature to preserve *Bombax ceiba* (simbal) wood. Wood of *B. ceiba* was sliced in to stakes of 13cm×5cm×2cm size. Three concentrations, 12.5, 25 and 50%, of castor oil were made in both the solvents. Dipping method was applied to treat wooden chunks of *B. ceiba* with each concentration. After application and compensating hygroscopicity, chunks were exposed to the termites' infestation in concrete soil pits prepared as test arena. Chunks were placed for termites' exposure after weighing treated woods were kept for considerable time period not less than one month and afterward chunks were carried to Termites Research Laboratory and were cleaned with brush to remove dirt and dust. Weight loss before and after exposure to termites was determined on laboratory weight balance. Weight loss% was noted to be very high in control treatments of all experiments. Comparing the efficacy of castor oil in both solvents, %weight loss was measured less for 50% concentration of castor oil in ethanol, >50% moisture content at 72hours of dipping interval. The data obtained from above experiments were analyzed using Minitab 17 software in Randomized Complete Block Design because of field condition. In experiment No. 1 the weight loss at 50% concentration was minimum in ethanol solvent (23.03%) and in water+detergent (26.60%). The maximum weight loss% was observed for control (untreated) group of both solvents (45% and 45.45%) respectively. Application of castor oil was done in experiment No.2 by the best concentration of castor oil in solvents after reducing the moisture contents of simbal wood up to <50% and >50% in oven drying method. Simbal wood was seasoned to reduce moisture contents less than 100%. Wooden stakes with >50% moisture contents was found with minimum weight loss% (24.53%) in water, (29.36%) in ethanol solvents and for <50% weight loss (14.43%) in water and (19.03%) in ethanol solvent was found at 50% of concentration of castor oil for dipping interval of 72hours. In experiment No. 3, simbal chunks were oven dried at varying temperatures (100, 150 and 200°C and exposed to termites' attack after weighing. Wood treated with highest temperature 200°C was noted with minimum weight loss (20.01%) in water solvent, (15.49%) in ethanol solvent at 50% concentration of 72hrs dipping interval.

NATURAL RESISTANCE OF DIFFERENT *MORUS* SPECIES AGAINST SUBTERRANEAN TERMITES**Zarnab Fatima, Sohail Ahmed and Sania Iqbal***Department of Entomology, University of Agriculture, Faisalabad, Pakistan*

Different wood species has natural resistance against termites. Wood of Genus *Morus* species was selected. Natural resistance of red and white mulberry woods was studied against the subterranean termites. In all experiments treatments were arranged Completely Randomized Design (RCD). The obtained data from all above experiments was analyzed by using Minitab 17 software. Results showed that under choice test different combinations were made and allow termite to attack. The weight loss of combination 1 (red and white mulberry) was minimum with 2.33%, combination 2 (Red mulberry and Poplar) was also minimum with 2.81% weight loss. Maximum wood consumption was observed in third combination (white mulberry and poplar) with 33.33% weight loss. Under no-choice test the red mulberry proved resistant against termites with minimum weight loss 1.50% as compared to other species. In another experiment, natural resistance of these wood chunks was compared with *Ficus religiosa*, another member of the Genus of the Moraceae family. Results showed that in choice test, the combination 1 (red and white mulberry) was more resistant with minimum weight loss (2.043%) as compared to other combinations. The red and white mulberry was more resistant than *F. religiosa*. Similar results were found under no choice test. Results from comparison of un-extracted and extractive free woods showed that extractive free wood was more susceptible to termites than un-extracted wood. In choice test, the extractive free wood of both white and red mulberry was vulnerable to termites. It can be seen from the comparison that weight loss in un-extracted wood was minimum as compared to extractive free wood. Wood chunks of different species of Genus *Morus* along with popular wood were placed in the field during April-June, July-September and October-December. After exposure, minimum weight loss in white mulberry was during April- June time period with weight loss of 3.07%. In the July-Sep the weight loss was 3.343%. Maximum weight loss was during the time period Oct-Dec with the weight loss of 4.03%. Minimum weight loss in red mulberry was during Oct-Dec time period with weight loss of 1.207%. In the April-June the weight loss was 2.233 %. Maximum weight loss was during the time period July- Sep with the weight loss of 2.387%. The moisture level of wooden chunks of genus *Morus* was reduced up to 50% and more than 50% by oven drying these chunks. When moisture was reduced <50% there was weight loss of 2% in white mulberry and 1.8% in red mulberry. When the moisture was reduced >50% there was more weight loss as compared to <50%. The weight loss at >50% moisture level in white mulberry was 8.7% and in red mulberry it was 2.8% the weight loss at >50% moisture level was due to evaporation of extractives.

STUDIES ON EXTENT OF PREVENTION OF WOOD FROM TERMITES BY IMPREGNATION WITH MORINGA SEED OIL**Huda Zahid, Maria, Sohail Ahmed, Sajid Abdullah* and Muhammad Jalal Arif***Department of Entomology, University of Agriculture, Faisalabad**Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad*

For the current study, *Moringa* seed oil was selected to preserve *Bombax ceiba* wood. Wood of *B. ceiba* was sliced in to stakes of 13cm×5cm×2cm size. Based on Completely Randomized Research Design, these wooden chunks, after weathering and application of *Moringa* seed oil (*Moringa oleifera*) at concentration of 12.5, 25 and 50% in three replications, were buried in soil for exposure to termites. The data was analyzed to determine the best moisture level at which the weight loss should be minimum. The obtained data from all above experiments were analyzed using Minitab 17 software. In experiment No. 1 the weight loss at 50% concentration of *Moringa* oil was minimum with oil in petroleum solvent (10.74%) and maximum in water detergent (34.11%). In experiment No.2, application of moringa oil was done by the best concentrations of oil in solvents after reducing the moisture contents of simbal wood <50% and >50% of their initial moisture content. Moisture contents were reduced by oven drying. Simbal wood was seasoned to reduce moisture contents less than 100%. At 50% concentration of applied oil on wooden stakes with moisture reduced <50% i.e. having more than 50% moisture contents occurred greater weight loss %age: (39.97%) in water emulsion and (26.5%) in petroleum emulsion and wooden chunks having less than 50% moisture i.e. moisture reduced >50%, undergone minimum weight loss: (32.62%) in water emulsion and (15.45%) in

petroleum emulsion. In experiment No. 3, simbal wood chunks were oven dried at different temperature regimes (100, 150 and 200°C) and exposed to termites' attack after weighing. Wood dried at highest temperature 200°C and treated with 50% oil emulsion in petroleum for 72 hours, suffered minimum weight loss (28.48), similarly 50% oil emulsion in water got minimum weight loss (39.85%) as compared to other two concentrations. These results indicated that as wood consumption was proportional to the degree of drying temperature. Then the chunks were placed for termites' exposure after weighing. Treated woods were kept for considerable time period not less than one month and afterward chunks were carried to Termites Research Laboratory and were cleaned with brush to remove dirt and dust. Weight loss before and after exposure to termites was determined on laboratory weight balance. Weight loss% was noted to be very high in control treatments of all experiments. Comparing the efficacy of castor oil in both solvents, %weight loss was measured less for 50% concentration of castor oil in ethanol, >50% moisture content at 72hours of dipping interval.

COMPARATIVE EFFICACY OF OILS AND INSECTICIDES COMBINATION AGAINST COTTON JASSID (*Amrasca devastans*) UNDER FIELD CONDITIONS

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A number of insect pests attack the cotton crop at its various growth stages and decrease its productivity in Pakistan. The insect pests with sucking mouthparts are reported to be more notorious due to their heavy infestations during the past few years. Cotton Jassid (*Amrasca devastans*) and whitefly (*Bemisia tabaci*) are dominating the sucking insect pest group attacking cotton crop in Pakistan. The use of chemical insecticides had gained much popularity due to quick response but the ill-advised applications had resulted in various problems including pesticides resistance development in insects and hence the incomplete control. The current research was focused to determine the combined effect of different oils and insecticides against the jassids on two Bt cotton varieties (FH-118 and FH-Lalazar). Four oils obtained from Neem, Castor, Linseed and Sesame plants along with a Mineral oil (Aliphatic hydrocarbons 97% W/V) was tested as combination with five insecticides, viz. diafenthuron, imidacloprid, profenofos, acetamiprid and spirotetramat at a ratio of 1:1. Three replications was designed under Split-plot Design and the experiment was conducted at the Research Area under the Department of Entomology, Youngwala, University of Agriculture, Faisalabad. Three sprays was done on weekly bases and data was collected after 1st, 3rd, 5th and 7th day of each spray. 26 combinations including control were used and the results revealed that Diafenthuron and Spirotetramat with Mineral oil showed the maximum population control of jassid. However, Acetamiprid and Profenofos with oils remains less effective to control jassid population but significant as compared to control treatment.

PREVALENCE OF DIPTERA TEPHRITIDAE SPECIES AS A MAJOR GUAVA FRUIT PEST AT FRUIT ORCHARDS OF LARKANA DISTT, SINDH

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Larkana District is an agricultural sector and known as pioneer city of Sindh Province. Variety of crops and fruits are produced and supplied towards various parts of the country. The most important commercial fruit is Guava, Different varieties of guava are grown and export to other cities as well as to foreign countries. In the Larkana guava fruit is attacked by fruit flies, mealy bugs and aphids. The fruit fly, (Diptera: Tephritidae) genus *Bactrocera*, is one of the most vicious pest of many pliable fruits and vegetables around the world. Fruit flies attack fruit trees, vegetables and not only reduce their yield but also affected the quality. Crop loss varies from a few per cent to 100% depending on fruit fly population, locality, variety and season. The present study started in the month of July 2017 to November 2018. 14465 fruit flies were captured through bottles filled with the methyl eugenol a sex pheromone from the guava orchards of three different localities of district Larkana namely village Sher Muhammad Jamali, village Choharpur

and village Naudero and sorted out into genus *Bactrocera* along with two species namely *Bactrocera zonata* and *Bactrocera dorsalis*. They have central job in guava orchards of different places of district Larkana and above genus is first time recorded from three different localities of Larkana district. Different guava varieties like Thadharami, Malto, Golo, Riali, local sindhi, and golden guava were taken to observe infestation rate of fruit fly species. Out of six most commonly growing guava varieties, we identify that the two guava varieties **RIALI** and **LOCAL SINDHI** are more prone to pest populations. While other varieties like Golden guava, Thadharami, Malto and Golo, have more resistance against tephritidae fruit flies species.

ENTOMOPATHOGENIC FUNGI AN EFFECTIVE MICROBIAL AGENT AGAINST LOCUSTS AND GRASSHOPPERS

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From last few years significant attention has been paid towards the utilization of many microbial agents' viz: Bacteria, Protozoa, Virus, Fungi and Nematodes against the many pest species of grasshoppers and locusts in world including Pakistan. During the present study an attempt has been made to investigate the lethal effect of entomopathogenic fungi i.e. *Beauveria* species. For this purpose about 1075 samples were collected amongst these 438 showed clean evidence of *Beauveria* infection but less numbers were infected by *Metarhizium*. Beside this, insect behavior after the treatment of this fungus was also changed. Overall, our results indicate that after application significant and decline level of infection in the field as well as in laboratory was noticed. Furthermore, in this study we have also documented: how biological control option could be incorporated into integrated pest management (IPM) strategies and what future and development work is necessary to implement such IPM strategies in Pakistan.

CONTROL STRATEGIES FOR OUTBREAK THREATS OF DESERT LOCUST DUE TO INSECURITY AND CLIMATIC CHANGE IN RECESSIVE AREAS

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Desert locust *Schistocerca gregaria* has been unexpected, abrupt emerging, destructive pest in various countries of old from ancient times. Various strategies have been applied for control management of desert locust such pesticides and other cultural and physical control. Moreover, modern technological tools like GPS, GIS, and Satellite data have been utilized to control locusts. However, somewhere these strategies failed to prevent swarm of desert locust due to insecurity in some countries. Beside this, global climate is changing day which also increased the threats of sudden outbreaks. In this regard a study carried out in Thar Desert to identify natural enemies of desert locust. It was observed that desert locust were preyed by various species of birds *Clanga hastate*, *Francolinus pondicerianus*, *Acridotheres tristis*, *Chlamydotis undulate*, *Corvus splendens*, *Mirafra erythroptera*, reptiles *Chamaeleo zeylanicus*, *Hemidactylus frenatus*, *Varanus bengalensis*, *Echis carinatus* and other arthropods such as Praying mantis, Thread waisted wasps and Spiders. But population of these natural predators was very low in fields. Present study suggests that introduction and proliferation of these natural predators in recessive breeding areas of desert locust can be an effective control strategy to mitigate the outbreak threats. Moreover, awareness is needed to local farmers about the role of natural predators in control of this pest. If saturated population of natural predators is maintained in the fields, desert locust would be in control without any field operation which is difficult during insecurity in areas having cold war.

PREVALENCE OF INSECT PESTS IN SUGARCANE FIELD FROM DISTRICT DADU SINDH PAKISTAN

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Sugarcane plays vital role in the field of Pakistan's agricultural economy, in Pakistan the industrial crop of sugarcane is occupying 1128.10 thousands /ha, yearly production of 63718.52 thousand/ tons. In terms of the sugarcane crop the lands of District Dadu is so fertilized and gives sugarcane in rich amount. The sugarcane crop is fertilized on about 13 thousand acres of the Dadu District lands and the annual production of sugarcane is commonly 20 thousand tons but this production is not sufficient due to attack of many insect pests at its different growing stages so for proper estimation of damage present study is designed. During the present investigation extensive weekly survey have been carried out into 08 sectors i-e Payaro, Patt Shareef, Kakar, Phulji, Buriri, Sade Mosani, Makhdom Bilawal and Sita where 04 varieties of sugarcane i-e SPF-234, CPF-237, BL4 and Thatta-10 are growing here. During the field survey significant large number of insects have been seen in the field that were, Stem Borer (*Chilo partellus*), Internode Borer (*Chilo sacchariphagus indicus*), Shoot Borer (*Chilo infuscatellus*), Root Borer (*Emmalocera depressella*), Black Bug (*Cavelerius excavates*), White Woolly Aphid (*Ceratosiphum lanigera*), Leaf hopper (*Pyrilla perpusilla*) and Thrips (*Fulmekiola serrata*) these pests causes usually damage to quantity, quality and also reduce the sucrose of cane.

INCIDENCE OF FRUIT FLY *BACTROCERA* SPECIES IN GUAVA FRUIT FROM DISTRICT SHIKARPUR, LARKANA

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Fruit fly (Diptera: Tephritidae) species cause infestation on different fruits. It is responsible for low yield and bad quality of fruits in Pakistan. Especially in Sindh province district Shikarpur and its Taluka Gari Yasin. Numerous species of Tephritidae family are observed to attack and damage the quality of fruit. Genus *Bactrocera* are considered as serious pest of fruit in Sindh Pakistan. They effect on the productivity that produce low yield from 50% to 80% in sense of quality and quantity of fruit. During present study a total population of *Bactrocera* species were counted 11050 from November 2017 to March 2018. District Shikarpur 5390 (34%) and its Taluka Gari Yasin 5660 (30%) major infestation ratio was found in guava fruit in month of March 11% out of 100 in guava fruit. Infestation ratio of fruit fly depends upon the humidity and temperature and kept different fruit orchards throughout the year from emerging flower to maturation of fruits.

THE COMPARATIVE STUDY OF INFESTATION OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN GUAVA AND MANGO ORCHARDS OF DISTRICT NAUSHAHRO FEROZE SINDH

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The study was conducted to observe the level of infestation of fruit flies in common fruits such as mango and guava in orchards of district Naushahro feroze during 2016 and 2017. Most delicious and tasty fruits were selected for infestation of fruit fly population in located area. The experiment was conducted in different areas of district Naushahro feroze such as guava and mango at Bhiriya city. The infestation data caused by fruit flies were recorded at fortnight intervals for guava and mango orchards. Data were recorded on counting fresh and infested fruits randomly from each at trial unit. The trials were laid out in randomized complete block design replicated three times. Findings

of the study showed that fruit fly infestations gradually increase from mid-April and reached its peaks in mid-June and July; whereas the fruit fly infestation dropped from the end of September to end of November in guava orchards. The infestations increased from mid of October thereafter started declined to mid of January. It was concluded that the presence of fruit fly was recorded in both orchards throughout their crop seasons and this pest with alternative hosts present whole the year. This is considered a critical one in the management of fruit fly.

MANGO LEAFHOPPERS OF PAKISTAN

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Mango is an important cash crop of Pakistan and plays vital role in export of Pakistan, due to its commercial importance it is largely cultivated in Sindh and Punjab and the crop is attacked by large number of insect pests. Pest management practices can not be undertaken without accurate identification. The leafhoppers of subfamily Idiocerinae are serious pests of mango in the Indian subcontinent where 10 genera and 43 species are recorded (Viraktamath, 2007). These leafhoppers can be recognized by their wedge-shaped appearance with short and broad head, ocelli on the face, facial sutures extending beyond the antennal pits almost to the ocelli, forewing with wide appendix and male genitalia with the style elongate and the connective rather T-shaped. A checklist of Idiocerinae from Pakistan is also provided. From Pakistan fauna, six species have been recorded from the region including three new species by Ahmed *et al.* (1980), *Amritodus saeedi*, *Idioscopus karachiensis* and *I. freytagi*, all breeding on Mango from Karachi. Further leafhopper collecting in the region has revealed a new species of *Tasnimocerus* Ghauri, *Idioscopus nagpurensis* (Pruthi) is newly recorded from Pakistan and *Idioscopus freytagi* and *I. karachiensis* are placed as junior synonyms of *I. nitidulus* (Walker). The audience will have insight to the finding of idiocerinae from Pakistan, with their information on habitus and other distinguishing characteristics.

USE OF *BRASSICA* TO ENCOURAGE THE POPULATION OF *EPISYRPHUS BALTEATUS* FOR BIOLOGICALLY CONTROLLING THE VEGETABLE PESTS

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Vegetable are heavily attacked by number of crop pests belonging to class Insecta, of them aphids, jasad and thrips are one of the most important phytophagous insects. They severely attack the different vegetable thus causing heavy loss in annual reduction of valuable crops and most importantly the vegetable which are having direct nexus with human health. Beside this one of the biggest problems of the world is environmental problem and UNO in general budget spends million dollars running different environmental projects. In this regard world is switching form use of hazardous chemicals to biological control and integrated pest management for controlling these crop pests. Present study was aimed to manipulate one of the important ecological factor for the encouraging the population of biological control agents (*Episyrphus balteatus*) in order to control the population of different vegetable pests. In this regard the different forms of cabbage, tomatoes, chilies, onions and spinach, containing brassica as secondary crop was visited systematical for the collection and observation of pests. The abundance of *Episyrphus balteatus* and aphids was measured after collection as well as in the field containing brassica, the correlation of abundance on vegetables containing brassica and those which don't was compared. Which showed there was strong positive correlation between the abundance and appearance pattern of *Episyrphus balteatus* in relation to brassica campestris. However there were other factors which must kept in mind before manipulating these biological control agents commercially in different vegetable. Collected data clearly shows that if we deliberately use brassica in different vegetable that will be proved fruitful in order to control the vegetable pests specially aphids, as brassica is attracting *Episyrphus balteatus*, while *Episyrphus balteatus* are the active predator of aphids.

MONITORING OF ADULT WHITEFLIES FLIES (*BEMISIA TABACI*) ON TOMATO PLANTS IN TALUKA TANDO MUHAMMAD KHAN, SINDH PAKISTAN

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Tomato (*Solanum lycopersicum*) is one of the most important vegetables in the world formally known as poor's man orange. Tomatoes are rich with minerals vitamins, essential amino acids, sugars and dietary fibres. Tomatoes are short durational plant gives a high yield. Tomato mainly infected by the whiteflies (*Bemisia tabaci*) by direct and indirect damage to the tomato plants. Whiteflies are polyphagous and feed on 600 different host plants. The present study was carried out on five different localities of taluka Tando Muhammad Khan namely Digh mori, Bijr Khan Talpur, Rajo Nizamani, Bhai Khan Talpur, and Shaikh Bhirkio from December 2017 to August 2018. The statistical data was collected from four plots per acre by counting the number of adult whiteflies (*Bemisia tabaci*) on five randomly selected plants followed by five randomly selected leaves per selected plant with the interval of 15 days. The result of the present experiment revealed that the maximum population of adult whiteflies above 8/leaf was recorded from June to August 2018. This is above the threshold level and caused 75% infestation in the leaves of Tomato plants and decreases the yield product of the tomato fruit.

ASSESSMENT ON RADIATION INDUCED STERILITY AND LONGEVITY OF *Aedes Aegypti* (DIPTERA: CULICIDAE) STRAIN OF SWAT, PAKISTAN

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Dengue scenario in Pakistan has changed from non-existing status to endemic during the last 30 years. The country has experienced seven mild and three severe dengue outbreaks over the past history of 30 years. As there is no effective vaccine available at the time, the use of an eco-friendly integrated vector control approach like Wolbachia based cytoplasmic incompatibility (CI) and Sterile Insect Technique (SIT) has gained much attention for vector control all over the world. Experiments in lines with the sterile insect techniques on sterilization of the male *Ae. aegypti* (pupa) of Swat strain was conducted using radiation doses of 0, 30, 60, 70, 90 105 (Gy) from gamma source (Co⁶⁰) at the Nuclear Institute for Food and Agriculture Peshawar, Pakistan. Post-irradiated pupae were allowed to emerge in standard adult cages and mated with virgin females of the same strain in 1:1 ratio. Male longevity and female fecundity was recorded from groups receiving the same doses of radiation. Observations on the number of eggs laid by females and their hatch rates along with their longevity were recorded for all treatments including control. An average of 71 eggs per female were laid by each female in control groups with 86 percent hatch rate. Females crossed with irradiated males of 30 Gy dose produced an average of 78 eggs per female with hatch rate of 30 percent. Other females in groups treated with 60, 70, 90 and 105 Gy produced 60, 42, and 38 and 69 eggs/female, respectively with hatch rate of 17 percent for 60 Gy and 0 with doses ≥ 70 Gy. Induced sterility of 100% was achieved with a minimum target dose of 70 Gy. Significant reduction in longevity was also observed for irradiation higher than 30 Gy doses. In conclusion, the Swat strain of *Ae. aegypti* can completely be sterilized with 70 Gy gamma irradiation for successful application of SIT for Aedes in Pakistan. Moreover, our results offer substantial support for developing SIT-based strategies for population suppression of Aedes vector mosquitoes in Pakistan.

ADJACENT NON-CROP VEGETATION AS A HABITAT OF RODENTS IN AGRICULTURAL LANDSCAPE OF POTHWAR PLATEAU, PAKISTAN

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This study was conducted to examine the significant role of adjacent non-crop vegetation in sustaining rodent populations. In a 14-month study, vegetation analysis was conducted by employing quadrat method to record the

vegetation around the rodent active burrows which provide habitat and cover to the rodent species during non-crop period. The dominant wild vegetation recorded from field boundaries that were covering the rodent burrows included *Cynodon dactylon*, *Saccharum griffithii*, *Dactyloctenium aegyptium*, *Dichanthium annulatum*, *Desmostachya bipinnata*, *Imperata cylindrical*, *Ziziphus nummularia*, *Achyranthes aspera*, *Calotropis procera*, *Sorghum halepense* and *Capparis decidua*. The year-around population of rodents in the Pothwar agro-ecosystem were maintained by the wild vegetation on the field boundaries, which provides shelter/cover and food. Rodent's community structure and richness have been related to variables such as habitat structure and complexity, temperature, rainfall, crop productivity, predation, trampling and grazing, surrounding landscape and succession of the natural wild vegetation. The livestock grazing, cutting, harvesting (for fuel wood, animal feed) and burning of field boundary vegetation is a common practice in the study area. During cropping season, local farmers did not manage the wild vegetation's since they have lack of environmental education and awareness about vegetation importance and wildlife conservation. The outcomes of this study suggest research and investigations on testing various ecologically-based rodent management strategies e.g. management of non-crop habitats, cleaning of crop cache in post harvested fields, management of wild natural vegetation providing food and cover during non-crop seasons that would seem essential to maintenance of habitats for rodent species in agricultural landscapes.

INTERACTION OF WHITEFLY, *BEMISIA TABACI* (GENNADIUS) AND ITS NATURAL ENEMIES AMONG HOST PLANT

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Bemisia tabaci (Gennadius) is destructive polyphagous pest of cotton which has showed the highly inter crop movement, high resistances to pesticides and vector of cotton leaf curl virus (CLCuV). In this review, we summarized interaction between whitefly and natural enemies (predators and parasitoids). Numerous parasitoids and predators attack on *B. tabaci*. Many studies showed well designed and appropriate time of conservation and augmentation process can make protected from the exposed crop. The technique effect from the many factor like release number, host availability, inter and intra competition, and stages of *B. tabaci* like nymph population as well as environmental factors. In future, some biological control strategies will be adopted for the suppression of *B. tabaci* because its attack found on lower leaf of plant and insecticide does not reached target location.

MANGO HOPPER POPULATION FLUCTUATION IN RELATION TO ABIOTIC FACTORS IN TEHSIL JATOI DISTRICT MUZAFFARGARH

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Mango is an important fruit in Pakistan and it is known as the king of fruits due to its sweet taste and nutritional value. However, the mango production is still low, mainly due to attack of numerous insect pests. Among the pests, mango hopper is the most harmful for the mango that causes major decline in fruit production. To assess the presence of this notorious pest in orchards, this study was conducted. Population fluctuation of the hopper in mango orchards was observed from February to August, 2016 in Tehsil Jatoi, District Muzaffer Garh. Data was recorded on fortnightly basis from renowned mango cultivar i.e. Chaunsa. Results revealed that hopper population was maximum in the second fortnight of April and minimum during the month of August. Population of mango hopper showed positive correlation with temperature and negative with relative humidity. Thus, the study will help in understanding the population dynamics in the different months of the year with environment.

ECONOMIC IMPORTANCE AND BIOLOGICAL CONTROL OF WHEAT APHID IN SOUTHERN PUNJAB, PAKISTAN

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Wheat (*Triticum aestivum* L.) is a staple food for more than 35% of the world population and is the main cereal crop of Pakistan. It plays major role in improving the economic stability of our country. In Pakistan it constitutes 60% of the daily diet of common man. The experiment was conducted in Multan to find out the effect of wheat aphid on yield of wheat grains. The variety was Faisalabad 2008. Infested spikes were tagged with red ribbon and non-infested spikes with blue ribbon. Non infested spikes were covered with butter paper. These spikes were regularly monitored daily. The results showed that grains in infested spikes were greater in number as compared to non-infested spikes but less in weight. There was very little effect on yield of wheat. Natural enemies like lady bird beetles, lacewings, hoverflies, and parasitoids were very active during wheat season. Use of pesticides result in mortality of natural enemies. Natural enemies are enough to control wheat aphid. Present study concluded that as compared to other pest control strategies bio control agents provides very effective control of wheat aphid and this is environmentally safe. These bio control agents help to reduce the aphid population from reaching the economic injury level and save farmer expenses. It is recommended to avoid use of insecticides to conserve natural enemies and to protect wheat from health hazards.

COMPARISON OF DIFFERENT INSECTICIDES AGAINST PINK BOLLWORM (*PECTINOPHORA GOSSYPIELLA*) OF COTTON IN LABORATORY CONDITIONS

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Pink bollworm is a notorious pest of cotton, and now a day it becomes a major challenge for many cotton growing areas of the world. For control of this pest, various chemistries of insecticides are being used in various countries. Likewise, in Pakistan many insecticides have been utilized for control of PBW. Continuous use of similar insecticides results in development of resistance in PBW against these chemicals. Best practice to delay resistance in insect pests is avoidance of similar molecules of pesticides. To assess effectiveness of various pesticides, current study had been devised. Triazophos, Leufenoron, Cydiox and the Emamectin Benzoate have been tested to assess their effectiveness against PBW in Lab conditions. The bioassay was performed by using flower-dip method in 25 \pm °C and 65 \pm 5 % RH conditions. Results revealed that Cydiox is most effective after 24 hours, followed by Leufenoron, Emamectin benzoate and Triazophos. Results obtained from the study can be employed in management programs against PBW in cotton.

ROLE OF DIFFERENT SOWING TIMES IN ECOLOGICAL PEST MANAGEMENT OF *LEUCINODES ORBONALIS* IN BRINJAL CROP

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Brinjal shoot and fruit borer (*Leucinodes orbonalis* Guenee) is the major insect pest of brinjal crop causing severe yield losses and different management practices are adopted for its timely control to avoid economic losses.

Use of synthetic insecticides is the common practice to manage it below threshold level which posing serious threats to humans and environment stressing the need of alternative pest management. We planned to utilize the ecological approach through different sowing times for possible long-term control of *L. orbonalis* and safety to the environment. These studies were carried out at University Research Farm, Koont near Chakwal following randomized complete block design with four treatments (1st, 20th of March and 10th, 30th of April) with four replications each. Brinjal variety "Nirala" was used with planting distance of 45 cm on ridges with distance of 75cm between rows and weekly brinjal shoot and fruit borer damage was recorded up to 28 weeks and crop yield was calculated. Maximum plant height (73cm) was gained by crop transplanted in start of March with maximum number of shoots (45.3/plant), squares (180/plant), flowers (144/plant), immature fruits (5.75/plant), mature fruits (3.75/plant) and fruit weight (86 grams). Maximum yield loss (84.6%) was observed in 30th April transplanted crop. Our results suggested that early crop transplant for brinjal was less damaged and more productive to avoid early pest infestation and yield losses.

EVALUATION OF PROGENY OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: CHRYSOMELIDAE) TO INFEST FOUR TYPES OF STORED PULSES

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Callosobruchus maculatus (F.) is one of the major notorious pest of economically important edible pulses during field and storage throughout tropical and sub tropical world including Pakistan. This species is spread throughout the world with the trades and other crops. *C. maculatus* can infest pods of pulses in the field and seeds in stores. The larvae of this beetle are the destructive stage which causes considerable damage and weight loss to the pulses by their feeding activity inside the seeds. A laboratory study was conducted to investigate the biotype (population) of *C. maculatus* at Zoology Department University of Sindh Jamshoro during August to November 2018. Green gram, black gram, cowpea and chickpea (kala chana) were used in the study. Five pairs of freshly emerged adults were introduced to the 500 g of tested pulses. The culture was maintained at 25-28 °C and 60%-90% relative humidity. The result indicate that the maximum number of population were found on green gram 2070 followed by cowpea 1926 and the minimum number of population were observed on black gram 1395 followed by chickpea (kala chana) 1432. On the basis of adult emergence here all the Hyderabad commercial tested pulses were highly susceptible to the strain of *Callosobruchus maculatus*.

INFESTATION OF TICKS (PARASITIFORMES: IXODIDAE) ON BUFFALOES AND COWS IN LARKANA, SINDH, PAKISTAN

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A study was designed to investigate infestation of ticks (parasitiformes: ixodidae) on buffaloes and cows in Larkana district of Sindh. Various dairy farms were visited fortnightly. The evaluation in this regard was carried out from March to September 2017. 1750 buffaloes and 84 cows were observed carefully in which 1093 buffaloes and 45 cows were infested with ticks (parasitiformes: ixodidae) and infestation rate was recorded 62.94% in buffaloes and 52.94% in cows. 4555 ticks (parasitiformes: ixodidae) of buffaloes and 931 ticks (parasitiformes: ixodidae) of cows were immediately preserved in glycerin filled bottles after removal from animal body. Collected ticks (parasitiformes: ixodidae) were brought in Entomology laboratory, University of Sindh where they were identified under binocular dissecting microscope. Species of genus *Hyalomma*, *Ixodes* and *Rhipicephalus* were recovered in massive quantities.

ROLE OF *WOLBACHIA* IN PEST MANAGEMENT: AN OVERVIEW

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Wolbachia are maternally inherited intracellular endosymbiotic bacteria which are present in almost 60% of entire insect species. *Wolbachia* is responsible to exploit different lethal host phenotypic responses i.e. parthenogenesis, feminization, Male-Killing and Cytoplasmic incompatibility. These vertically transmitted bacteria (endosymbionts) live within the body of the partner by a symbiotic association. Exponential growth in the identification of endosymbionts suggesting novel control strategies. These lethal abilities also make the *Wolbachia* able to be the component of Bio-control programs and in management of disease transmitting insects. The present review is an effort to provide successful insights as an alternative approach to resistance management in field insects and management of human disease transmitting-vectors.

DEVELOPING EFFICIENT TRAPS FOR CATCHING MOSQUITOES

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In this study we designed mosquito traps with different bait solutions. Basically two types of traps were designed i.e., three simple traps (Type-.1, Type-.2 and Type-.3) and three modified miniature traps (Type-.1, Type-.2 and Type-.3). In each type of trap we used a different bait. The traps were installed in the fields and their efficiency to attract and trap the insects was recorded. In each modified miniature trap we attached a different colour LED light to attract the insects. Our results showed that modified miniature traps with red LED were most effective as they captured maximum number of insects and the best chemical bait was brown sugar and yeast solution. It was concluded from the study that these modified traps can be further improved and used for mass trapping of mosquitoes and other insects.

EVALUATION OF MOSQUITO OVITRAPS IN RURAL AND URBAN HABITATS IN MULTAN

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Mosquitoes are vectors of deadly diseases like malaria and dengue. They breed in stagnant water. Rapid changes such as deforestation, monoculture, un-controlled urbanization, building dams and diverting rivers, all create conditions that allow mosquitoes to breed. Among various surveillance methods, installation of ovitraps is a common and effective method to monitor population fluctuations of various species of mosquitoes especially dengue vectors. Considering this scenario, the experiment was conducted to evaluate the efficacy of mosquito ovitraps in various location in urban and rural areas of Multan. Each trap was monitored weekly to determine the presence of mosquito larvae. The collected specimens were preserved in 70% ethyl alcohol for identification purpose. The highest value of ovitrap larvae was found from nurseries, near water channels, shades and human residences. The dominating genus found in ovitraps was *Culex*. Thus, the study will help in the management of vectors and in identifying the best ovitrap installation sites for mosquitoes.

EFFICACY OF DIATOMACEOUS EARTH AND *BEAUVERIA BASSIANA* AGAINST RED FLOUR BEETLE (*TRIBOLIUM CASTANEUM*) AND GRANARY WEEVIL (*SITOPHILUS GRANARIUS*)

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Red flour beetle and granary weevil are among the serious insect pest of stored products. The high infestation of these insect pests cause huge qualitative and quantitative losses to cereals. Currently, Diatomaceous earth (DE) and Entomopathogenic fungi (EPF) have been registered in some countries for long-term control against a range of stored grain insect pests. The aim of this study was to determine the efficacy of DE and EPF against red flour beetle and wheat weevil in order to provide effective control of these insect pests. Application of DE (100 ppm or 200ppm) and EPF (2.6×10^6 conidia ml⁻¹ or 2.6×10^7 conidia ml⁻¹) alone and in combinations were used. The insect samples of red flour beetle and granary weevil were collected from different storage facilities of district Layyah followed by colony establishment at IPM laboratory of Department of Agriculture Entomology, BZU Bahadur Sub Campus Layyah. Lab Bioassays were done and data was recorded at 5, 10 and 15 days interval to evaluate the effectiveness of treatments involved while control (untreated wheat) was also maintained. Highest mortality of both the insect pests (red flour beetle and granary weevil) was recorded in treatment (DE @ 200ppm and EPF @ 2.6×10^7 conidia ml⁻¹) while it was lowest in treatment (EPF @ 2.6×10^6 conidia ml⁻¹). In conclusion, DE and EPF in combination (at higher doses) can be used for effective management of these two important stored grain insect pests.

REARING OF PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (LEPIDOPTERA: GELECHIIDAE) ON ARTIFICIAL DIET

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Pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) is a destructive pest of cotton worldwide. It causes reduction in seed cotton yield, oil content and normal opening of bolls. In the current study, life cycle of this pest was studied on artificial diet at Central Cotton Research Institute, Multan, Pakistan. The major contents of diet include cotton seed flour, chickpea flour, sucrose, agar agar and distilled water. The newly emerged larvae were singly transferred to glass vials containing a piece of artificial diet. The vials were placed in laboratory at 29°C temperature and 60-65% relative humidity. After pupation, pupae were shifted to a glass jar until adult emergence. The adult (male and female) pair were placed together for mating and egg laying in glass jar containing cotton swab soaked in 20% honey solution as adult diet. The results showed that eggs hatched in 4 days and larval period was completed in 16-20 days with a total of four instars. Pupal period was 9-10 days and adults remained alive for 4-5 days. These findings will be helpful in designing pest management strategy for this destructive pest.

INFESTATION OF *BEMISIA TABACI* ON COTTON CROP IN DISTRICT SANGHAR, SINDH, PAKISTAN

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The silver leaf whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) is a polyphagous insect attacking many plant species of economic importance. Present study was conducted from May to October in cotton growing

areas of district Sanghar. Infestation of white fly started in June and reached its maximum rate during July and August. The population recorded above ETL.

EFFECTS OF NEEM OIL AGAINST STORE GRAIN BEETLES *TRIBOLIUM CASTANEUM* AND *TRIBOLIUM CONFUSUM*

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Store grain beetle are serious pest of many store products specially cereals and are very difficult to control. Neem oil is a bio pesticide and now in these day normally used for control of many insect pest. Present study was carried out in laboratory from July 2018-November 2018. Store grain beetles were reared on wheat grain on different temperature. 20 beetles were introduced in each jar. 1, 2, 3 and 4 ml concentrations of neem oil were made and used separately in each jar to observe the effect of neem oil. During present study there was no any significant mortality of beetles were observed against neem oil.

EVALUATION OF NEUROTOXINS AGAINST SCAVENGING TERMITE, *ODONTOTERMES OBESUS* (RAMBUR) (BLATTODEA: TERMITIDAE: MACROTERMITINAE)

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The Scavenging Termite, *Odontotermes obesus* (Rambur) (Blattodea: Termitidae: Macrotermitinae) is an important higher termite which cause damage to many crop plants and wood structures. This species is difficult to control with existing baiting systems that use chitin synthesis inhibitors as bait active-ingredients. In the current study, we evaluated three neurotoxins (fipronil, imidacloprid and thiamethoxam) for their suitability as bait active-ingredients along with their optimal dose for use in baits against *O. obesus*. Five doses of each active-ingredient (causing >0 % and <100 % mortalities) were prepared in water. No-choice bioassays were conducted in glass petridishes using treated filter paper technique. The results showed significant differences among three neurotoxins after 48 h. Fipronil was comparatively more toxic with lower LC₉₀ value (9.16 mg/l) followed by thiamethoxam (15.11 mg/l) and imidacloprid (23.15 mg/l). Workers showed erratic movement in petridishes having filter papers treated with imidacloprid and thiamethoxam while they showed normal behavior in petridishes having filter papers treated with fipronil. The results suggested that imidacloprid and thiamethoxam are not suitable for use in baits but can be used as soil termiticides. While fipronil can be used effectively in baits and as soil termiticides for the management of termites in agriculture crops and buildings.

EFFECT OF SYNTHETIC SALICYLIC ACID ON WHITEFLIES

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Cotton is one of most important fiber and cash crops of Pakistan. It contributes about 1.0 percent share in GDP and 5.5 percent share value added in agriculture. Whitefly has been ranked one of the most destructive and serious

pests of cotton crop. Whitefly de-saps the leaves by sucking it and secretes honey dew on leaves which leads to development of black mold (sooty mold) and also causes CLCuV diseases. Salicylic acid (SA) is a phytohormone. Generally, salicylic acid concentration is increased by the attack of phloem-feeding insect attack. Synthetic salicylic acid when applied to the crop at 1mM concentration, it reduced the number of whiteflies significantly. Synthetic salicylic acid induced the resistance in plant and repelled the whiteflies. When synthetic SA applied on plants, the concentration of the volatile compounds like terpenes were increased as compared non treated plants.

ECOLOGICAL IMPACT OF PB ROPES ON *BEMISIA TABACI* AND BENEFICIAL FAUNA OF COTTON

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Excessive amount of insecticides destroys natural enemies of cotton pests and consequently the population of whitefly flares up. The present research focused on the use of PB ropes to reduce application of insecticides and to investigate the ecological impact on *Bemisia tabaci* and beneficial fauna in cotton fields. Two cotton varieties (BS-15 and NIAB 878) were sown in research area of Muhammad Nawaz Shareef University of Agriculture, Multan during 2018 in 30 acres. PB ropes dispensers (PB-ropes L[®]) (Shin Etsu Ltd. (Tokyo, Japan) were installed @120/acre at pin head square stage of cotton and was compared with untreated check. It was observed that application of PB ropes reduced need for insecticidal applications, thus helped conservation of beneficial fauna in cotton fields throughout the season, which kept whitefly populations below EIL. Population of whitefly was recorded less (2.45/leaf-seasonal average) in PB ropes treated fields as compared to untreated check (8.63/leaf-seasonal average). Furthermore, higher population of green lacewing (8.31/plant) was observed in PB ropes treated field. It was concluded that populations of whiteflies were kept below EIL due to conservation off green lacewing. This positive effect of PB ropes is presumably due to the action of the natural enemies of cotton insect pests. This indirect effect of mating disruption (PB ropes) is of great value within the framework of IPM in cotton.

POPULATION OF GREEN LACEWING (*CHRYSOPELRA CARNEA*) ON COTTON CROP SOWN ON DIFFERENT PLANTING TIMES IN MULTAN

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Cotton crop is damaged by a number of insect pests with varying population densities throughout the growing season. A field experiment was performed to observe the population of green lacewing (*Chrysoperla carnea*) on cotton crop, which was sown on different planting times (during April, May and June, 2018) at Multan. Maximum population of green lacewing was recorded from early planted crop (April sown) during the early vegetative phase (1.729/leaf) and up to the start of flowering (1.33/leaf). On the plants of May sown crop, population of *C. carnea* was found moderate and remained around 0.042-0.521/leaf during early vegetative phase to the start of flowering. Whereas on plants of late sown cotton (June), population of *C. carnea* was found comparatively low on flowering stage. It was further observed that population of *C. carnea* was found continuously during the months of August and September from cotton plants sown on different planting times. It is concluded that timely sown cotton in April has additive advantage with comparatively higher population of *C. carnea*, which remains in the field from early vegetative stage to late flowering stage of the crop during the season. This continuous presence of predacious *C. carnea* has a significant effect on insect pests population especially whitefly.

EFFECT OF SILVER NANOPARTICLES ON THE VIABILITY OF FRUIT FLY, *DROSOPHILA MELANOGASTER*

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Silver nanoparticles are nowadays widely used in commercial products and they also reduce the efficiency of living system. Thus, toxicity levels of silver nanoparticles against living organisms needs to be investigated. In this study silver nanoparticles were synthesized from reduction of silver by plant leaves extract of Citrus limon and were assessed for their toxicity against the in vivo model *Drosophila melanogaster*. Scanning electron microcopy (SEM) of AgNPs suggested that they were uniform in size and well dispersed in the solution. Fourier-transform infrared (FTIR) spectroscopy indicated the presence of functional groups involved in bio-reduction and the surface plasmon resonance band of UV-Vis spectra showed that stable silver nanoparticles were formed. Toxicity assessment of AgNPs on *Drosophila melanogaster* at higher concentrations i.e. 70% and 100% showed high mortality rates of 40-50% and 42-55% for larvae and adults, respectively. Developmental delay was observed at higher concentration of AgNPs (100%) with an increase in duration of from 73.19-123.17 hours and 181.44-240 hours for larvae and adults, respectively. Scores of wing venation and wing spots also deviated from control groups at higher concentrations of AgNPs. Adults showed normal pigmentation at 20%, light pigmentation at 50% and 70% but non-pigmented cuticle was observed at 100%. A concentration dependent decrease in fecundity of flies was also observed. It was concluded that higher concentrations of silver nanoparticles was toxic against *Drosophila melanogaster*. Further, it was suggested that genotoxicity and dose dependent effect of AgNPs on morphology of insects should be assessed.

EFFICACY OF PHOSPHINE FUMIGATION IN VARIOUS PLASTIC PACKAGING MATERIALS AND FOOD SUBSTRATES AGAINST LARVAE OF *TRIBOLIUM CASTANEUM* AND *TROGODERMA GRANARIUM*

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Plastic packaging provides a barrier to insects and micro-organisms from getting access to food besides maintaining the internal temperature. In order to see the effect of different packing materials and food substrates on efficacy of phosphine fumigation against *Trogoderma granarium* Everts, khapra beetle (Coleoptera: Dermestidae) and *Tribolium castaneum* Herbst, red flour beetle (Coleoptera: Tenebrionidae), an experiment was conducted at the Entomological Laboratory of the University College of Agriculture and Environmental Sciences, The Islamia university of Bahawalpur. Insects were reared on natural diet at optimum conditions of 30±2 °C and 65±5 % relative humidity. Three packing materials i.e. polyethylene, polypropylene, polyvinylchloride and four food substrates were used i.e. wheat, rice, mung bean and black gram for *T. granarium* while wheat flour, rice flour, corn flour and oat flour for *T. castaneum*. The effect of different packing materials on percent mortality of *T. granarium* and *T. castaneum* was significant at 24 and 48 hours after the treatment while the effect of food substrates on percent mortality was significant at 48 hours for *T. granarium* and 24 hours for *T. castaneum*. The maximum percent mortalities of 53% and 85% were recorded in *T. granarium* at 24 and 48 hours in all the food substrates packed in polyethylene and polypropylene. Similarly the maximum percent mortalities of 81% and 96% were recorded in *T. castaneum* at 24 and 48 hours in all the food substrates packed in polyethylene and polypropylene. It was concluded that polyethylene and polypropylene are the most suitable packing materials for various food substrates in order to control *T. castaneum* and *T. granarium*.

TOXICITY OF SYNTHETIC INSECTICIDES AND NEEM OIL AGAINST BIO-CONTROL AGENTS OF COTTON MEALYBUG, *PHENACOCCLUS SOLENOPSIS* TINSLEY (STERNORRHYNCHA: PSEUDOCOCCIDAE) UNDER LAB CONDITIONS

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Mealybugs are small, soft-bodied, plant sucking insects and their common name is due to the waxy material which covers the bodies of adult females. Studies were carried out to find out the toxicity of four chemical insecticides viz. commando (97% DF), confidor (20% SL), lannate (40% SP), actara (25 WG) and neem oil at various concentrations against the larvae of *C. carnea*, adults of *B. suturalis* and *A. bambawalei* through residual contact under laboratory conditions. Surface treated bioassay method was used for the evaluation of toxicity levels of four synthetic insecticides and neem oil. All the four tested insecticides caused significant mortality of the beneficial bio-control agents of *P. solenopsis*. According to the measured values commando was the highly toxic and actara was moderately toxic insecticide to tested bio-control agents of *P. solenopsis*. Neem oil was found to be harmless to larvae of *C. carnea* ($LC_{50} = 247.06 \text{ mL.L}^{-1}$) and adults of *B. suturalis* ($LC_{50} = 144.35 \text{ mL.L}^{-1}$) but slightly harmful to adults of *A. bambawalei* at higher doses. Interestingly, *A. bambawalei* males ($LC_{50} = 22.19 \text{ mL.L}^{-1}$) were more susceptible to neem oil than females ($LC_{50} = 33.82 \text{ mL.L}^{-1}$). Based on LC_{50} values it was concluded that neem oil is safe to the bio-control agents of *P. solenopsis* and can be used in compatible with integrated pest management programs.

**EFFICACY OF NANOPARTICLES AGAINST RICE WEEVIL *SITOPHILUS ORYZAE* (L)
(COLEOPTERA: CURCULIONIDAE)**

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Rice weevil (*Sitophilus oryzae* L) is a primary insect pest of the stored grains like rice, maize and wheat. The quality and quantity of products decrease by severe infestation of the pest. An experimental study was carried out to check the efficiency of the nanoparticles like nanosilica, nano alumina and nano clay at three different concentrations @ 350, 250, 150 ppm kg^{-1} against pest, *Sitophilus oryzae* at Muhammad Nawaz Shareef University of Agriculture, Multan in 2018. These nanoparticles were used to control *Sitophilus oryzae* under laboratory conditions. All the treatments were replicated thrice. Among the treatments, nanosilica at 350 ppm kg^{-1} dosages gave maximum mortality at one day after application followed by 250 and 125 ppm kg^{-1} which cause mortality at three days after treatment. The study resulted that nanosilica @ 250 and 150 were highly effective, cause 80.12 and 71.33 percent mortality, respectively after one day of treatment and gave complete control after three days. The oviposition and adult development of *S. oryzae* reduce with nanosilica treatment. The nanoparticles damage the protective coat of insect cuticle, resulting death of pest. This nanoparticles technology had great potential in pest management.

BIOLOGICAL CONTROL OF *BEMISIA TABACI* IN COTTON

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Cotton an important crop in Pakistan. It is also known as the White Gold because of high economic returns. The cotton crop is attacked by the chewing and sucking pests thus lowering its production. Among the sucking pests, White fly is one of the destructive pest of cotton. White fly (*Bemisia tabaci*) is a polyphagous pest with high reproduction rate. Also the same pests is reported to be one of the resistant pests against the insecticides. Beside, affecting the crop, it spreads cotton leaf curl virus (CLCV) among the different crops. Several studies have been conducted against *Bemisia tabaci* to understand its relation with environment, host plant, viral transmission, development and survival but a very few studies has been reported on biological control. The documented predators of white fly are *Eretmocerus eremicus*, *Encarsia formosa*, *Amblyseius* spp. *Typhlodromus* spp. and lacewings, *Chrysoperla carnea*. All were recorded feeding on *Bemisia tabaci*. But the most efficient predators are *Eretmocerus eremicus* and *Encarsia formosa*. Thus, it can be concluded that both of these can be used in bio control programs of white fly management.

TRAPPING EFFICIENCY OF HIGHLY EFFECTIVE SLOW RELEASED FORMULATIONS OF BIODEGRADABLE WAXES WITH METHYL EUGENOL AGAINST *BACTROCERA ZONATA*

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Experiment was carried out to evaluate the performance of highly effective Slow-Released Formulations (SRF) of Methyl eugenol with Lanolin wax, Candellila wax, Bee-wax, Carnauba wax and Paraffin wax in orchard of University of Agriculture Faisalabad, Pakistan against fruit flies. The waxes were mixed with methyl eugenol in 1:9 ratios. The results revealed that SRF of Candellila, Paraffin, Bees and Carnauba wax attracted 13.77, 11, 8.15 and 7.23 flies/day/trap which was 2.6, 2, 1.5 and 1.4 times higher than standard respectively and exhibited 41.42%, 32.05%, 20.98% and 12.87% attractive index respectively, proved moderately attractive slow-released formulation to *B. zonata* and was categorized as Class-II slow-released formulation (AI = 11 - 50%). However, SRF of Lanolin wax trapped 1.81 flies/day/trap which was 3 times less than standard and exhibited -61.86% attractive index proved little or non attractive slow-released formulation and was categorized as Class-I slow-released formulation for *B. zonata* (AI < 11%).

MANAGEMENT OF STORED SEED PEST

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To monitor stored pests, variety of techniques has been developed. These are the biological, chemical and the physical management tools. The stored pest is divided into two types. One of them is the pest which directly harms the seed while the other pest lives inside the food residues near facilities. Thus, there is a need to develop some of the new techniques which can give better assessment about the pest population. Thus, in this analysis the latest methods to control the pest population at marketing level has been provided and also the basis that which technique is more appropriate in accessing the pest population. In this, economic threshold level was developed at the different levels of storage commodities. The different levels are processing plants, and retail business. Thus taking enough samples ((20–30 samples) will present enough information to access that weather the population is low or above the economic threshold.

EXPLOITING THE LARVICIDAL POTENTIAL OF INDIGENOUS PLANT EXTRACTS AGAINST *AEDES AEGYPTI*

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Aedes aegypti, act as the major vector for many human diseases such as dengue fever, dengue hemorrhagic fever, chikungunya etc. causing millions of deaths every year. Due to the insecticidal contamination of the environment and behavioral resistance in vectors, increasing attention is now being focused on environmental friendly control methods. The use of easily degradable plant-based compounds are considered to be one of safest, cheapest approach of insect pests control especially dengue vectors as an alternative source for the synthetic chemicals. Therefore, the present study was made to monitor the larvicidal properties of various different plant extracts against third instars larvae of *Aedes aegypti*. These extracts were applied at three different dosage rates i.e., 1, 2 and 3% and 1-2 days of exposure time under laboratory conditions. The results of the present study showed that the black pepper (100% mortality) after 24 hours exposure period at 1% concentration. Similarly, garlic has shown the mortality effect as 56.66% at 24 hours exposure time at 3% concentrations. When the dosage rate and exposure time of the plant extract was increased, the mortality rate was also amplified. After 48 hours of exposure time, the garlic has shown significant mortality effect of (80-90%) at 3% concentrations. Similar mortality trend was observed in mint extracts. While even at the highest dosage rate i.e 3% of onion extract showed the minimum mortality as (13.33%) after 48 hours exposure time. In the control treatment only (3%) mortality was found at 48 hours of exposure. The efficacy in term of mortality were found in order of black pepper > garlic > mint > onion. Thus environment friendly plant based pesticides can be integrated with other available techniques for population suppression of larvae and can be successfully included the dengue vector control program.

BEHAVIORAL CONTROL OF PINK BOLLWORM BY PYRETHROID: A REVIEW

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Pink bollworm *Pectinophora gossypiella* is the most serious pest due to losses in both yield and quality. Many tactics are used to suppress the population of the pink bollworm. Chemical control is responsible for the mortality of insect but sub-lethal dose also disturb the behavior of the insect. Chemical communication interrupted by applying the sub-lethal dose of the pyrethroid between male and female of *Pectinophora gossypiella*. Studies show that applying the insecticide much less than LD50 reduced the calling ability in female adult of the pink bollworm. Female lost the ability to calling the male and it cause mating disruption. Male also affect by the sub-lethal dose of the pyrethroid, although male recover from its effect but their signal capturing ability is reduce due to blockage of pheromones sensor organ. So, studies show that insecticides may not only limited to eliminate and potentially killing of the insect but also low doses may be part of the management tactic. In future, this is path of research area for the scientists to make sub lethal dose as a part of the integrated pest management because minimum chance of insecticide resistance.

IMPACT OF NUTRIENTS AND ENDOPHYTIC ENTOMOPATHOGENIC FUNGI MANIPULATION ON APHID-PARASITOID INTERACTION

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The aim of present research was designed to investigate the impact of nutrients and endophytic entomopathogenic fungi manipulation on aphid-parasitoid interaction. Three formulations of entomopathogenic fungi

[*Metarhizium anisopliae* (MA), *Beauveria bassiana* (BB) and *Verticillium lecanii* (VL)] was applied by two methods (foliar application and soil-dressing methods) at three concentrations (1000 ppm, 2000 ppm and 4000 ppm) prepared in water. Mixtures of different nutrients were prepared and applied along with the entomopathogenic fungi fifteen days before the release of aphids on plants as described in methods and material. Three experiments (one for MA, 2nd for BB, 3rd for VL) laid out in completely randomized design in which treatments were randomized and repeated thrice. The maximum mortality of aphids of entomopathogenic fungi MA, BB and VL was observed (66.3%, 61.13% and 53.83%) and the maximum reproductive potential (nymphs produced/female) of VL, BB (33.16%) and MA (31.16%) when aphids feed on cabbage plants manipulation 4000ppm of nutrients by foliar application and The maximum percent attraction of released parasitoids of entomopathogenic fungi MA, BB and VL (100%) when aphids feed on cabbage plants manipulated 1000ppm with nutrients by foliar applications and by soil-dressing methods 0%. Maximum percent survival rate (nymph-adult) of aphid of entomopathogenic fungi MA, BB and VL (14.7%, 12.73%, 9.63%) and the maximum percent parasitism of parasitoid of entomopathogenic fungi of BB and VL shown by results (100%) while MA was observed (97.2%) and the maximum percent parasitism of parasitoid of entomopathogenic fungi of MA, BB and VL shown by same results (100%) manipulated with 4000ppm with nutrients.

COMPARATIVE EFFICACY OF VARIOUS INSECTICIDES AGAINST MANGO THRIPS

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Mango (*Mangifera indica* L) is the widely grown. It is known as the 'King of Fruits'. However, mangoes are being attacked by the complex pests but mango thrips is one of the important pests. Both nymphs and adults feed on the young and newly emerge leaves of plants, leaving the scars through which after maturity fruits become unmarketable. An experimental study carried out by in Muhammad Nawaz shareef University of Agriculture Multan, during 2018. The mango orchard was visited and pest scouting was done. Data was taken from 10 fresh leaves of mango. During the study three insecticides like Bifenthrin, Imidacloprid and Acetamiprid was used. The concentrations are 60, 100 and 100 doses per 100 liter of water respectively with three replications. The data has been recorded after 12, 24, 72 hrs. The results showed that the population was low. Among the insecticides, Acetamiprid showed maximum control than other two insecticides. Acetamiprid gave about 90.12 % success efficiency followed by Imidacloprid (85.23%) and Bifenthrin (81.01). thus, the said pesticides can be used effectively in the management of thrips.

QUANTIFICATION OF PESTICIDE RESIDUES IN COW MILK SAMPLES OF SARGODHA, PUNJAB, PAKISTAN

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Raw cow milk samples from citrus plantation zone in district Sargodha were analyzed for the presence of Deltamethrin and Malathion pesticides using HPLC. Results showed that 100% of milk samples were contaminated with pesticide residues. Malathion and deltamethrin were detected in milk samples at the mean concentration of 0.47µg/kg and 0.54µg/kg respectively. None of the samples showed concentration higher than MRL prescribed by FAO. These results are of concern as adverse health effects exerted by deltamethrin and Malathion are well known.

COMPARATIVE EFFICACY OF VARIOUS INSECTICIDES AGAINST MANGO THRIPS

Immad Anwar, Faheem Aslam, Muhammad Ramzan*, Hasnain Abbas, Masood Maqbool, Ghulam Murtaza, Muhammad Adnan and Muhammad Jamshed Iqbal

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Mango (*Mangifera indica* L) is the widely grown. It is known as the 'King of Fruits'. However, mangoes are being attacked by the complex pests but mango thrips is one of the important pests. Both nymphs and adults feed on the young and newly emerge leaves of plants, leaving the scars through which after maturity fruits become unmarketable. An experimental study carried out by in Muhammad Nawaz shareef University of Agriculture Multan, during 2018. The mango orchard was visited and pest scouting was done. Data was taken from 10 fresh leaves of mango. During the study three insecticides like Bifenthrin, Imidacloprid and Acetamiprid was used. The concentrations are 60, 100 and 100 doses per 100 litter of water respectively with three replications. The data has been recorded after 12, 24, 72 hrs. The results showed that the population was low. Among the insecticides, Acetamiprid showed maximum control than other two insecticides. Acetamiprid gave about 90.12 % success efficiency followed by Imidacloprid (85.23%) and Bifenthrin (81.01). thus, the said pesticides can be used effectively in the management of thrips.

EFFECTS OF PROTEIN HYDROLYSATE AND FOOD SUBSTANCES IN THE MANAGEMENT OF FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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Fruit Flies (Diptera: Tephritidae) are major pests of different fruits and vegetables of many countries including Pakistan. In the country, about 55 % loss is caused in fruits and vegetables by the fruit flies. Thus to manage the fruit flies, a study on its management was conducted. In the study, efficiency of different substances was checked. These are Protein hydrolysate and other different food substances like Banana, Sugar Solution and honey. In the study, multiple insecticides were also mixed with these substances. The used insecticide is bifenthrin and coragen was used. Data was recorded for the two times per week. Results revealed that protein hydrolysate is the most preferred by the fruit flies. On an average, 7.5 adults visited the substance per 30 minutes. While the lowest flies was recorded on honey solution. The study will help in the better management of fruit flies.

ROLE OF INSECT POLLINATION IN IMPROVING PHYSIO-CHEMICAL PROPERTIES OF LITCHI FRUIT (*LITCHI CHINENSIS*)

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Litchi (*Litchi chinensis*) is the 2nd most important fruit crop of the world after mango. It contain bioactive compounds that have improving health benefits. Pollination is one of the most important ecosystem service provided by insects as it contributes 35% to global food volume. Worldwide, only few studies have evaluated the effectiveness of pollination in improving physico-chemical properties of vegetable and fruit crops. Therefore, current study is planned to investigate the role of insect pollination in improving the physico-chemical properties of litchi. Honeybees

(*A. florea* and *A. dorsata*) and Syrphid flies (*Ischiodon scutellaris* and *Sphaerophoria begalensis*) were among the dominant floral visitors in litchi flowers while maximum abundance of insect pollinators was recorded at 08:00 am. Moreover, significant difference was observed in physical (fruit length, fruit diameter, fruit weight, Pulp weight and seed weight) and chemical (TSS, pH, and Vitamin C) parameters between insect pollinated and caged fruits. Conserving honey bees and syphid flies may enhance litchi yield leading to the improved socio-economics of the farming community.

EVALUATION OF SINGLE AND COMBINED APPLICATIONS OF *BEAUVERIA BASSIANA* AND THIAMETHOXAM AGAINST DIAMOND BACK MOTH (*PLUTELLA XYLOSTELLA*) UNDER LABORATORY CONDITIONS

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The present study was conducted to evaluate the efficiency of *Beauveria Bassiana* against Diamond back moth (DBM) and the compatibility with Thiamethoxam under laboratory conditions. Ten leaves per disc DBM used for each treatment and cabbage leaf dip in suspension. After evaporation of excess water second larval stage of DBM were placed *B. bassiana* [Bb (1), Bb (h)], and Thiamethoxam [Thi (1) and Thi (h)] were applied to larval 2nd instar of DBM. Single and integrated effect of *B. bassiana* and Thiamethoxam were observed. The highest mortality (>80%) was observed in 2nd instar larvae when exposed to combination of both agents [Bb (1) + Thi (h)]. Contrarily lowest mortality (<50%) was recorded in 2nd instar larvae when treated with lowest concentration of Bb (Bb-1) 7 days after treatment. The integration of *B. bassiana* at higher concentration, whereas Thiamethoxam at lower dose (Bb (h) + Thi (1)) was found more lethal to DBM larvae. This signifies the need of combining *B. bassiana* and Thiamethoxam that can reduce the cost of management with least harm to environment.

REPELLENT ACTIVITY OF SOME ESSENTIAL OILS AGAINST *CALLOSOBRUCHUS CHINENSIS* L. (COLEOPTERA: BRUCHIDAE) FOR THE SAFETY OF CHICKPEA SEED

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To improve the quality and safety of foods, chemical methods to control grain pests have been replaced by alternative methods. Chickpea is an important cash crop of Pakistan. Among many notorious pests, *Callosobruchus chinensis*, causes more damage during post storage. The aim of this study was to determine repellent and toxicant effects of different essential oils extracted from plants of tulsi (*Ocimum tenuiflorum*), neem (*Azadirachta indica*), deodar (*Cedrus deodara*), marigold (*Tagetes erecta*), eucalyptus (*Eucalyptus saligna*) and taramira (*Eruca sativa*). Repellent effect of these oils measured by olfactometer through cotton swab and grain coating method. Toxic effect and reduction of oviposition of these oils on chickpea was also determined. Among all six essential oils, highest repellency was showed by eucalyptus oil and lowest by taramira oil than others at 50% concentration with cotton swab method. While maximum repellency was obtained by neem oil followed by deodar oil, eucalyptus oil, marigold oil, taramira oil, and tulsi oil during grain coating method at 95% concentration. During mortality test we found that deodar oil coated chickpea has significant mortality rate and also have the highest effect on fecundity of *C. chinensis*.

DEVELOPMENT AND FIELD EVALUATION OF ECOFRIENDLY IPM MODULE AGAINST SHOOT AND FRUIT BORERS (NOCTUIDAE: LEPIDOPTERA) OF OKRA (*ABELMOSCHUS ESCULENTUS* L.)

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Okra (*Abelmoschus esculentus*) is an important summer vegetable of Pakistan. Okra shoot and fruit borers (*Earias vittella* and *Helicoverpa armigera*) are the most devastating pests causing substantial loss to the okra produce. Farmers primarily rely on irrational and recurrent use of synthetic insecticides which, apart from unsatisfactory control of target pests, pose serious threats of environmental contamination and health hazards. Therefore, this three years field study was aimed to screen out available okra germplasm against infestation of *Earias* spp. and selective insecticides and botanical extracts and from the most effective treatments to develop and evaluate a bio-intensive IPM module against shoot and fruit borers of okra. From 12 okra genotypes, Sabzpari was the most resistant/tolerant one with minimum shoot and fruit infestation (13.49 and 9.34%, respectively), while Pusa Swani appeared to be most susceptible okra genotype with maximum shoot and fruit infestation (39.57 and 23.16%). Maximum shoot and fruit infestation by *Earias* spp. was observed in mid-June while minimum values were recorded for end May and early July dates. According to proximate analyses of okra fruits, all components were negatively but weekly correlated except crude fat contents which were significantly and negatively correlated with the percent fruit infestation. Among insecticides, emamectin benzoate and indoxacarb and among botanicals neem (*Azadirachta indica*) and bitter apple (*Citrullus colocynthis*) were the most significant treatments which resulted in maximum reduction of *Earias* spp. infestation and larval population and increased okra marketable fruit yield by 45, 44, 61 and 41%, respectively as compared to control treatments. The most resistant/tolerant okra genotype (Sabzpari) and the most effective insecticides and botanical extracts were integrated in IPM (bio-intensive) module along with egg cards of *Trichogramma chilonis* and cultural and mechanical control methods and were compared with farmers' routine practices module and control module. Maximum shoot and fruit infestations (19.69 and 16.90%) were recorded in control module (M3) and minimum ones (7.15 and 3.74%) were found in IPM (Bio-intensive) module (M2), while farmers' routine module exhibited intermediate values regarding *Earias* spp. infestation. Hence, bio-intensive (IPM) module is recommended to the indigenous okra growers against okra shoot and fruit borers and other lepidopterous pests of fruits and vegetables under the agro-climatic conditions of central Punjab, Pakistan.

INSECTICIDAL EFFICACY OF BIOSYNTHESIZED SILVER NANOPARTICLES, *B. THURINGIENSIS* AND TRIAZOPHOS AGAINST *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE)

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Pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is key pest of cotton worldwide. Traditional management measures used by farmers are proved worthless to effectively combat this notorious insect pest. This study was conducted on finding an effective alternate to conventional insecticides and to evaluate the efficacy of some non-conventional pest control agents i.e. silver nanoparticles (AgNPs), bio-formulation (*Bacillus thuringiensis*) and a commercial insecticide (Triazophos) under laboratory conditions. Biosynthesis of AgNPs was carried out through the chemical action of aqueous leaf extract of neem (*Azadirachta indica*). Synthesized AgNPs were characterized by UV-visible spectroscopy and Transmission Electron Microscopy (SEM) techniques. Single and combined efficacy of AgNPs, *Bt* and Triazophos was evaluated against 2nd instar larvae of PBW and mortality rate was recorded at different time intervals i.e., 24 hours, 72 hours and 120 hours. Results indicate that combined application of AgNPs+Triazophos (50ppm + 100 ppm) exhibited > 90% larval mortality as compared to other treatments. It was evident from the results that nanoparticles are efficient larvicidal agents for PBW control.

STUDY OF DELTAMETHRIN TOLERANCE AND TOXICITY OF BIFENTHRIN, CHLORPYRIFOS AND THEIR MIXTURES FOR THE MANAGEMENT OF INSECTICIDE TOLERANCE IN STORED GRAIN PEST, *TROGODERMA GRANARIUM*

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In Pakistan, Deltamethrin has been in use for the management of A₂ quarantine stored grain pest, *Trogoderma granarium* (Khapra beetle) for decades in grain storage sectors especially those are under PASCO. As resistance is an outcome of inappropriate and common practice of any insecticide therefore present study aimed at evaluating the Deltamethrin tolerance and efficacy of Bifenthrin, Chlorpyrifos and their mixtures for the management of three populations of 4th instar larvae of *Trogoderma granarium* collected from the PASCO godowns of DG Khan, Okara and Gujranwala while one Susceptible population (never been exposed to any insecticide) was used as a reference strain. Residual film method was followed for the toxicological analysis. The LC₅₀ values against Deltamethrin were 376.74, 1860.77, 1914.30 and 2017.45ppm for Susceptible, DG Khan, Okara and Gujranwala populations respectively. The level of tolerance in term of resistance ratio (RR) against Deltamethrin was 4.94, 5.08 and 5.36 for DG Khan, Okara and Gujranwala populations respectively. In case of Bifenthrin, the LC₅₀ was 359.32, 416.58, 524.01 and 598.91ppm for Susceptible, DG Khan, Okara and Gujranwala populations respectively. Against Chlorpyrifos, the LC₅₀ was 189.33, 305.37, 376.30 and 459.15ppm for Susceptible, DG Khan, Okara and Gujranwala populations respectively. The efficacy of Set I mixture combination (1:1) of Bifenthrin and Chlorpyrifos was intercessor between toxicities of Bifenthrin and Chlorpyrifos. Whereas mixtures of Set II such as 2:1, 3:1 (Bifenthrin: Chlorpyrifos) and Set III including 1:2 and 1:3 mixtures (Chlorpyrifos: Bifenthrin) were effective than Bifenthrin and Chlorpyrifos when tested alone against all three Deltamethrin tolerant populations of 4th instar larvae. In all the mixture treatment 1:3 mixture was found to be most effective and not only LC₅₀ values were recorded at very low doses but very strong synergism was also recorded even against most tolerant Gujranwala population of 4th instar larvae (LC₅₀, 123.63 ppm; RTU, 4.00). Results revealed that DG Khan, Okara and Gujranwala populations of 4th instar larvae were moderately resistant to Deltamethrin based on their RR values which showed that resistance was developing in them. The gradation of effectiveness for different treatments against all four populations of 4th instar larvae was Bifenthrin < Chlorpyrifos < 1:1 < 2:1 < 3:1 < 1:2 < 1:3. On the basis of this toxicological data 4th instar larvae of Gujranwala population were observed to be highly tolerant to all of the treatments among three populations. It is also concluded that insecticides should be used in rotation with insecticides belongs to different class or should be deployed in the form of mixtures because these are potential resistance controlling tool since they can magnify the toxicity of insecticides against resistant/tolerant insect pests.

IDENTIFICATION, BIOLOGY AND MANAGEMENT OF DIFFERENT SPECIES OF MANGO FRUIT BORER: A REVIEW

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Mango is known as the king of fruit. It is attacked by many insect and pest at different stages of bearing. Mango fruit borer was minor pest but now a days becoming a serious pest due to attack. The most destructive stage of this pest is larval stage. Two species are well known due to the mode of action. Dry twigs of the branches and soil inside brownish cocoon are preferred sites for the pupation of mango fruit borer. There are two reported species of mango fruit borer *Citripestis eutrapphera* and *Autocharis albizonalis*. For the identification of *Citripestis eutrapphera* the eggs are white and turn red after 1 day. Larvae are pale pink-brown, 4 bands of black spots along the body darken to red brown and the length of larvae is 15 mm. Pupae are dark brown with one pointed end. Adult forewings color is dark brown and hindwings are pale white-grey with a grey band around the margin. The wing span of female is 24 mm and male is 20 mm. For the identification of *Autocharis albizonalis* the eggs are Oval, waxy white, the larvae have brown head, white

body, red intersegmental band and the length of larvae is 12.3 mm. pupae are dark brown and slightly pointed. The head of moth is small brown with prominent brown snout and mesothoracic legs are yellowish in color, the forewings are ashy wood in color. The attack of this pest started from the pea sized fruit till maturity. This insect lay eggs on the site where two fruits are hanging together and touching each other. Female moth prefers the site for egg laying which is hide to sunlight and air. This pest is distributed in different areas of the world. Eggs are laid on the surface of fruit. This insect reduces the quality and nutritional value of mango fruit. First and 2nd instar feed on the surface of fruit skin and further lateral larval instars boring the fruits through tunneling. They feed on pulp of fruits hence damaging fruits quality. It could be managed by different practices like cultural control, which included removal of malformation, debris, biological control which include egg parasites (*Trichogramma chilonis*, *Trichogramma chilotraee*) and larval predators (*Rhychium attrisimum*, *Carcelia* (*Senometopia*)), physical control which include bagging and proper pruning and some other plant based pesticides which include Azadirachtin and Citronella Essential Oil.

FOXTAIL MILLET (*SETARIA ITALICA*) AS A FUTURE CROP UNDER CHANGING ENVIRONMENT. A REVIEW

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Crop production is considerably decreasing due to rapid change in environmental conditions globally. For instance, increasing temperature, salinity, and decreasing water availability are demanding such crops which are highly resistant against these changes and high in nutritional value. In the past many crops which are stress tolerant and highly nutritious than traditional cereals were neglected due to least research about such valuable crops. Foxtail millet (*Setaria italica*) which is a cereal contains all characteristics to flourish under changing climate and nutrient rich. It can ameliorate the food security of the increasing population, as it has essential amino acids, proteins, good dietary fiber, and antioxidants. Foxtail millet is an ancient crop of drylands but now is being cultivated in many countries at research bases. The aims of this review are to describe the impact of different abiotic stresses on foxtail millet, physiological adaptations of foxtail millet in the response of the abiotic stress and give the detail description of foxtail millet's nutrition.

FEEDING POTENTIAL OF *CHRYSOPERLA CARNEA* STEPHENS (CHRYSOPIDAE: NEUROPTERA) ON SUGARCANE WHITEFLY *ALEUROLOBUS BARODENSIS* MASK (ALEYRODIDAE; HOMOPTERA) UNDER LABORATORY CONDITIONS

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Sugarcane whitefly *Aleurolobus barodensis* has attained the status of economic pest in sugarcane. It damages the crop by extracting large quantities of phloem sap and transmission of diseases and severe whitefly infestation can reduce crop yield up to 50%. *Chrysoperla carnea* is a generalist predator that feeds upon variety of soft bodied insect pests in different crops like cotton, fruits and vegetables. It can be integrated as a control measures along with other control methods against whiteflies in sugarcane. Present studies were carried out to determine the feeding potential of green lacewing on whitefly nymphs to reveal its possible use as a biocontrol agent in sugarcane crop. Studies were conducted at temperature 24 °C, Relative humidity 65 percent and light and dark period of 10: 14 hours. Results showed that larvae of *C. carnea* consumed varied number of different whitefly nymphal instars. First instar predator larvae consumed 8.2, 5.8, 4.5 and 4.8 nymphs of *A. barodensis* whereas, consumption of second instar *C. carnea* was 18.6, 20.44, 32.26 nymphs. Third instar larvae consumed 40.25, 42.40, 48.5 and 60.40 nymphs. Over all predation of third instar was more on third instar nymphs of *A. barodensis*.

ECOLOGICAL IMPACT OF PB ROPES ON *BEMISIA TABACI* AND NATURAL ENEMIES OF COTTON INSECT PESTS

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Cotton (*Gossypium* spp.) is a fiber crop in all over the cotton producing countries. Pakistan is the 4th largest cotton producing country among the other major cotton growers in the world. Cotton whitefly, *Bemisia tabaci* (Gennadius) is the destructive pest of cotton which can destroys 66% of Punjab's cotton crop and also serve as vector of cotton leaf curl virus (CLCuV); the major threat to Cotton in Pakistan. This article reviews the effect of PB rope on whitefly population and its natural enemies. Mating disruption technique is effective against the pink boll worm (*Pectinophora gossypiella*) as well as indirect effect of PB rope on *B. tabaci* and natural enemies. Studies showed that lowest population of whitefly found in PB rope treated field and conserved its natural enemies. In this review, we summarized area wide management of whitefly and also check the effect of PB rope on whitefly and its natural enemies population. In future, this IPM strategy helpful for the better management of whitefly and reduce the less use of insecticides.

PREVALENCE OF HEAD LICE IN SCHOOL CHILDREN OF UNIVERSITY MODEL SCHOOL, ISLAMIA UNIVERSITY BAHAWALPUR

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The objectives of present study was to determine the overall prevalence of pediculosis in school children's and their relationship between sex, age groups, children's parent profession groups and income groups of hosts and parasite. Prevalence of head lice infestation was studied in University Model School, Islamia University Bahawalpur during January to March, 2018. Samples were collected from 209 school children. A standard questionnaire was designed for recording a variety of information at the time of survey. Visual inspection of student's head and combing of their hair for 2-4 minutes were made to check the presence of lice, nymphs and eggs. Out of 209 school children, 82 were boys and 127 girls. Out of 209, 43 (20.57%) were positive for pediculosis. Pediculosis was positive in 9 (10.97%) boys, and 34 (26.77%) girls with higher prevalence in girls. Pediculosis had highest prevalence i.e. in age group of 9-12 years. In conclusion gender showed non-significant ($P>0.05$) difference between girls and boys. But apparently girls showed higher prevalence as compared to boys. The age group (9-12 years) of host showed higher prevalence than age groups of (5-8 & >12), but statistically the difference was non-significant. Income groups and children parent's profession showed significant ($P<0.05$) influence on prevalence of lice.

OVER-WINTERED MONITORING OF PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) FROM DIFFERENT CARRYOVER SOURCES

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Pectinophora gossypiella Pink Bollworm (PBW) is a serious pest of cotton crop in Pakistan and causes 20-30% crop loss. In the month of November, its larvae diapause in left-over bolls and in soil. The present study was

conducted to monitor the diapausing larval population of PBW in ginning factories and cotton left over bolls on cotton sticks heaps in tehsil Multan and Kot Addu during 2015-16. The population of PBW was recorded by counting the larvae from one kg ginning waste from each factory while twenty bolls per cotton sticks heap were inspected on a weekly basis. In Multan and Kot Addu, the average larval infestation was 9.84 ± 0.25 & 4.67 ± 0.36 during 2015 and 8.75 ± 2.34 & 2.30 ± 0.04 during 2016 in left over bolls, respectively. The average larval population per kg ginning waste was 17.5 & 12.75 during 2015 and 15.25 & 7.8 during 2016 in Multan and Kot Addu, respectively. PBW is a monophagous pest and is difficult to control with insecticides due to larvae present inside bolls. During overwintering, it is mostly found in left-over bolls and cotton ginning waste. Cultural control like grazing of left-over cotton bolls, shredding of cotton sticks and complete destruction of cotton ginning waste may reduce its infestation on forthcoming cotton crop.

EFFICACY OF DIFFERENT NATURAL AND SYNTHETIC CHEMICALS FOR THE CONTROL OF *DROSICHA MANGIFERAE* G. (MARGARODIDAE: HEMIPTERA)

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Mango mealy bug (*Drosicha mangiferae* G.) is an important insect pest of mango fruit and belongs to the order hemiptera and family Margarodidae. Now-a-days it is creating a threat to successful mango production and causing serious mango losses. Due to its efficient colonizing behavior in different agro ecological zones of the globe, *D. mangiferae* became a major pest of different fruit trees and found to attack about 62 host plants. The present experiment was conducted to evaluate the efficacy of different insecticides and botanicals for the control of mango mealy bug. Eggs of *D. mangiferae* were collected from soil in the radius of 2 meter from stem of trees and then reared in laboratory. In first experiment five insecticides prophenofos, chloropyriphos, lambda cyalothrin, bifenthrin and deltamethrin were used. Leaf dip bioassay method was used, leaves of mango were immersed in the recommended dose rate solution of insecticides for the 10 s and then air dried in the room temperature for 30 minutes and then inserted in petri dishes along with moistened filter paper. Five insects were placed in each petri dishes. Efficacy of insecticides were tested on all 4 instars of mango mealy bugs. Data was recorded after 24,48,72,96 and 120 hours after treatment. Prophenofos was proved most toxic insecticide and resulted 100% mortality after 120 hours of treatment and bifenthrin was proved less effective than all other insecticides. While in second experiment botanical solution of 0.4, 0.6, 0.8 and 1 percent concentrations of Neem, Garlic and Turmeric were used in mortality test against adult female of *D. mangiferae*. Leaf dip bioassay were used and 10 Adult female mango mealy bugs were subjected to test in each treatment and data was recorded after 24, 72 and 120 hours of treatment. Results indicated that neem was proved most toxic botanical against adult *D. mangiferae*.

MANAGEMENT OF JASSID (*AMRASCA BIGUTTULA BIGUTULLA*) AND WHITEFLY (*BEMICIA TABACI*) ON BRINJAL CROP BY USING DIFFERENT INSECTICIDES

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Solanum melongena L. "Brinjal" is most important horticultural crop grown all over the world and mostly in all region of Pakistan. Initially it is cultivated in Thailand, Burma, India and China. In sub-continent, wide range grown regions are in India, China, Japan, and Pakistan. After brinjal shoot and fruit borer, the most destructive pests of this crop are (*Amrasca devastans* Distant) (Hemiptera: Cicadellidae) (jassid) or leafhopper and Whitefly (*Bemisia tabaci*) Gennadius, (Hemiptera: Aleyrodidae) which are sucking and notorious pest of the brinjal crop. In the light of above background, present experiment was conducted to evaluate the different selected insecticides against the jassid and whitefly on brinjal crop during year 2017 in spring season at the Research Area of Entomology, Department of Entomology, University of Agriculture, Faisalabad, Pakistan, by using randomized completely block design (RCBD). Four insecticides were evaluated against the pests for their effective management viz; Flonicamid 50WG

@60gm/acre, Imidacloprid 200SL@ 60ml/ acre, Buprofezin 25 WP @ 200gm/acre, Nitenpyram 10%AS @200ml/acre with four replications. Jassid and whitefly population was counted from upper, middle and lower leaf of the selected plants. Results were concluded that maximum mean population (2.68) was observed in control while among the treatments maximum mean population (1.78) were recorded on the plots treated with Buprofezin 25WP and minimum mean population (0.96) were recorded on the plots treated with Nitenpyram 10%AS against jassid at recommended field dose rates. In case of whitefly results slightly differ and showed that among treatments maximum mean population (2.51) were observed in Buprofezin 25WP while minimum mean population (1.45) were recorded in the plots treated with Flonicamid 50WG and highest mean population (3.92) recorded in control treatment. It is concluded that Nitenpyram proved best against jassid and Flonicamid against whitefly. So, these insecticides could be used against the management of jassid and whitefly on the brinjal crop.

IMPROVED DELIVERY SYSTEM OF *B. BASSIANA* THROUGH DEVELOPMENT OF FOAM FORMULATIONS AND COLOR PREFERENCES OF FRUIT FLIES TO METHYL EUGENOL TRAPS & INTEGRATION WITH MALATHION FOR MANAGING *B. ZONATA*

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Bactrocera zonata Saunders (Tephritidae: Diptera) is one of the major constraint for mango production in Pakistan. It deteriorates quality and quantity due to premature dropping. Yellow warning issued to Pakistan in 2013 due to fruit fly in export shipments. The rejection of mangoes due to the presence of maggots is the greatest threat which makes it unfit for human consumption. Present study was focus on the colour preference of *B. zonata*, their population dynamics and evaluating pathogenicity of entomopathogenic fungus, *Beauveria bassiana* and biorational insecticide malathion alone and in combination under laboratory and field conditions. Further the delivery of *B. bassiana* was improved by applying it in foam-mixed formulation. The study was conducted at Muhammad Nawaz Shareef University of Agriculture, Multan and mango orchard of Mango Research Institute, Multan by installing traps of 4 different colors in three replicates. This research work was done in the year 2018 from July to September. Pathogenicity of *B. bassiana* and malathion was tested on larval (L₂), pupal and adult stage of *B. zonata*. Growth and development (larval duration, pupal duration, adult longevity), mortality, pupation, adult emergence, eclosion, mycosis and sporulation was recorded. It was found that maximum populations of fruit flies (irrespective of species) were attracted to yellow (75.59±15.43 flies/trap/week) and green (71.68±13.27 flies/trap/week) colored traps. In the year, 2018, peak population was observed in the August. The species of fruit flies identified from mango orchards were *B. zonata* and *B. dorsalis*. *B. zonata* with occurrence of 89.14%-91.66% was highly dominant in mango orchard as compared to *B. dorsalis* with 8.33%-10.85% occurrence. From this experiment it can be concluded that *B. zonata* show more attraction to yellow and green colored traps, therefore timely installation of these traps is recommended as a part of integrated pest management for monitoring, detection and control of fruit flies.

MANAGEMENT OF EGGPLANT (*SOLANUM MELONGENA* L.) SHOOT AND FRUIT BORER (*LEUCINOES ORBONALIS* GUENEE) BY USING DIFFERENT INSECTICIDES

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Brinjal is a very popular and delicious vegetable cultivated in all regions of Pakistan as poor man crop. It is a rich source of minerals like copper, iron, sulphur, iodine, phosphorus and calcium and vitamin D, A& C. In Pakistan and India brinjal is attacked by more than 140 species of different insect pests like aphid (*Aphis gossypii*), white fly (*Bemisia tabaci*), coccinelide beetle (*Epilachna vigintioctopunctata*), jassid (*Amrasca bigutulla*), thrips (*Thrips tabaci*) and brinjal shoot and fruit borer. Above these mention pests BSFB is very notorious pest of this crop. Its

young larvae can infest four to seven fruits in its life cycle. The pest provide yield loss of crop up to 85-90% and also deteriorate the vitamin C contents of fruit. Peoples used many methods for its management like cultural control, biological control and chemical control but most reliable and effective method is chemical control. The chemical control is main practice for its management due its easy availability, easy to use, time saving and less labor requirement. Hence present research was performed to evaluate the effectiveness of the insecticides against the pest for its management. The experiment was performed at Entomological Research farm, Department of Entomology, UAF, Faisalabad. Punjab during year 2017 in spring season of sowing of crop. The experiment was consists of five treatments viz: T1 (Spinosad 240SC @ 60ml/ acre), T2 (Flubendiamide 48SC @ 50ml/acre), T3 (Bifenthrin 10EC @ 200/acre), T4 (Emamectin benzoate 1.9 EC @200ml/acre) and T5 (Control).The design (RCBD) was used for the allocation of treatments in field in which each treatment were replicate four times in the experiment. The application of insecticides were done after 14 days of interval and data of fruit and shoot infestation were taken after seven and fourteen days of insecticides application. The results showed that the minimum mean percent shoot infestation were recorded in T2 (Flubendiamide 48SC @ 50ml/acre) which was (9.5%) followed by T4 (Emamectin benzoate 1.9 EC @200ml/acre), T1 (Spinosad 240SC @ 60ml/ acre), T3 (Bifenthrin 10EC @ 200/acre) and T5 (Control) (11% 13.78%, 12% and 70%) respectively. Similarly minimum meant percent fruit infestation was recorded in T4 (Emamectin benzoate 1.9 EC @200ml/acre) which was (8.5%) followed by T2 (Flubendiamide 48SC @ 50ml/acre), T1 (Spinosad 240SC @ 60ml/ acre), T3 (Bifenthrin 10EC @ 200/acre) and T5 (Control) (10%, 13%, 14.5% and 68.5%) respectively. and maximum number of healthy fruits were also recorded in T4 (Emamectin benzoate 1.9 EC @200ml/acre) as compared to all other treatments.

POPULATION DYNAMICS OF SUCKING PESTS IN BRINJAL ECOSYSTEM IN RELATION TO WEATHER PARAMETERS

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Brinjal (*Solanum melongena* L.) is very important vegetable crop of many countries including Pakistan. It is attacked by many sucking insect pests, resulting reduction of yield and prohibiting growth of crop. An experimental study carried out under field condition to check the pest population relative to weather parameters, at MNS-University of Agriculture Multan, Punjab Pakistan during 2018. The three sucking pests like jassid (*Amrasca biguttula biguttula*), aphid (*Aphis gossypii*) and whitefly (*Bemisia tabaci*) were recorded in the study. The data regarding pest populations along with temperature and relative humidity was recorded twice in a week. The whitefly, aphid and jassid populations were 0.15 - 4.85, 0.1 - 1.25 and 0.85 - 6.25 per leaf with an average 2.5, 0.67 and 3.55, respectively. The jassid population was highest (6.25%) during last week of November and lowest (0.85%) at the start of month, while maximum population of whitefly (4.85/leaf) and aphid (1.25/leaf) was recorded during first week of November. It has been observed that sucking pest populations showed positive correlation with temperature. Both jassid and whitefly populations have significant correlation with temperature, while aphid population was found to be negatively correlated.

MANAGEMENT TACTICS FOR THE CONTROL OF PINK BOLL WORM *PECTINOPHORA* *GOSSYPIELLA* (SAUNDERS) IN COTTON

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Cotton (*Gossypium hirsutum* L.) is one of the most cultivated crop and ranked 2nd for oil content after Soybean in the globe. It is known as ‘White Gold’ for Pakistan. In GDP, it shares 1.0 %. As compared to production in

Pakistan. Due to insect pest damaged yield of cotton has decline in recent years. Pink bollworm known as destructive bollworm of cotton and its management is limited due to its feeding behavior and lack of knowledge at farmer's level. Pink bollworm management in cotton is one of the important IPM toll. The management tactics used for the control of *Pectinophora gossypiella* were the use of PB-Ropes, experiment was laid out a farmer's field, District Khanewal, Tehsil Jahania. The cultivars of cotton were IUB-2013, NS-181, MNH-996 selected for the installation of PB- Ropes. PB-Ropes were installed in 8 acres of each cultivar, hence total area covered with PB-Ropes were 24 acres. Installation of PB-Ropes completed in 2 steps. Total number of PB-Ropes were 135/acre. First we install 55 PB-Ropes/acre on 10th June 2017. The actual releasing capacity of pheromone by PB-Ropes was last for 90 days, after 90 days its pheromone concentration decrease day by day but it can be work last for 120 days, in second step we installed 80 PB-Ropes/ acre after 90 days of first installation of PB-Ropes. The data were recorded on daily basis. It was found that there were no infestation of PBW on bolls of Cotton where PB-Ropes were installed, Larval infestation was also minimum about 1% in PB-Rope field, while on the other hand in Control field infestation was high. For monitoring of Male moth we also installed sex pheromone traps (Hexadecadienyl acetate). Correlation of moth catches with temperature, light and relative humidity also monitored. The population of PBW Moths were zero in PB-Ropes field. Hence PB-Ropes give 95% control.

EVALUATION OF MICROBIAL INSECTICIDES AGAINST *BACTROCERA CUCURBITAE* FRUIT FLY UNDER LAB CONDITION

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Integrated Pest Management (IPM) have a significant importance for the control of insect pests and in improving sustainable agriculture without damaging the environment. Now a days, a new trend is develop to use the biological agents against insect pests which gives a positive response to overcome the pest population. These biological agents are insects, nematodes, bacteria or fungi. Studies shows that use of Entomopathogenic fungi can be effective approach against insect pests and it is environment friendly. Current study was concerned about the use of different isolates of EPF strains of *Beauveria bassiana* (Balsamo) Vuillemin, *Isaria fumosorosea* (Wize) and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) against the larvae, pupae and adults of *Bactrocera cucurbitae* under Laboratory conditions. Fruit flies were reared under laboratory conditions and isolates of EPF were also be cultured under laboratory condition according to appropriate methods and ingredients. After purification of EPF, three concentrations 1.5×10^8 , 1.5×10^7 and 1.5×10^6 were used for the inoculation of adult fruit flies. Larvae and pupae of fruit flies were inoculated through dip methods and were placed in petri dishes and topical method was performed on the adult to observe the mortality. Mortality data were observed after 1, 3, 5 and 12 days. Overall results were significant and *M. anisopliae* was highly effective against all stages of fruit fly. It caused 21 to 41% mortality against larvae and when these treated larvae were again exposed with EPF concentrations they caused 100% mortality. *B. bassiana* showed 51% mortality and that of *I. fumosorosea* caused 40% mortality. Results of present study showed that microbial insecticides have a potential to control fruit flies and also play a significant role in IPM to control insects pest.

EFFECT OF HYBRID AND CONVENTIONAL MAIZE VARIETIES ON BIOLOGY AND MORPHOMETRIC OF *CHILO PARTELLUS* (SWINHOE) (LEPIDOPTERA: PYRALIDAE)

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Maize stem borer, *Chilo partellus* (Swinhoe) is one of the major pests of maize causing a huge loss to production. The present study was conducted to assess the performance of hybrid maize with conventional maize in

reference to duration of biological life stages, length and weight of *C. partellus* in laboratory conditions. The results found that eggs hatched between 4-8 days on conventional maize and 4 to 7 days on hybrid maize. The total larval period ranged from 20 to 48 days on conventional and 18 to 42 days on hybrid maize. During its larval period the caterpillar moulted five times and had six larval instars. The mean average lengths of all larval instars ranged from 4.16 ± 0.32 to 29.89 ± 1.06 mm on conventional maize, while 3.22 ± 0.23 to 24.44 ± 0.11 mm on hybrid maize. The weights of larval instars reared on conventional maize were weighted greater than those on hybrid maize. The fully developed larva undergoes pupation in the larval tunnel with total duration of 5 to 8 days on both conventional and hybrid maize. The results for an average mean pupal length of 18.06 ± 0.06 measured on conventional maize and 16.67 ± 0.10 mm on hybrid maize. However, average mean pupal weight varied from 0.418 ± 0.019 g maximum on conventional maize and 0.352 ± 0.003 g minimum on hybrid maize. The longevity of female adults varied from 6 to 8 days on conventional maize and 4 to 8 on hybrid maize and for male adults it varied from 4 to 8 days on conventional maize and 4 to 6 on hybrid maize. The length of adult female varied from 16.20 mm to 17.77 mm on conventional maize, while 13.77 mm to 14.37 mm on hybrid maize. The weight of adult female reared on conventional maize varied from 0.834g to 0.902g, whereas, 0.704g to 0.832g on hybrid maize.

USE OF STICKY TRAPS IN EGGPLANT

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In eggplant (*Solanum melongena* L.) the management practices for insect pests rely heavily on scheduled insecticide applications. whitefly *Bemisia tabaci* that mainly infests eggplant, there was an urgent need to develop a sustainable control system, Integrated Pest Management (IPM) approach. Successful IPM is based on control options that are economically sound, environmentally acceptable and user friendly. Whiteflies use their piercing, needlelike mouthparts to suck sap from phloem, the food-conducting tissues in plant stems and leaves. Yellow sticky traps are a commonly used method for population monitoring of many pests. The experiment trial was conducted at Rice Research Institute Dokri, Larkana to evaluate the color attraction of White fly (*Bemisia tabaci*) through sticky cards in brinjal crop, cards were placed randomly in brinjal field.

POPULATION DYNAMICS OF MITES ON EGGPLANT IN RELATION TO ABIOTIC FACTORS

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Eggplant (*Solanum melongena* L.) is one of the popular vegetable worldwide and cultivated on large scale in multiple countries such as India, Bangladesh and in Pakistan. Regarding production of brinjal, Pakistan does not rank at the prominent position. It is because of pest complex most likely the mite fauna in brinjal. Thus, a study is designed to access the population availability of mites. The study was conducted in November 2018, under the field conditions. Data was collected for three times in a week and correlated with the environmental factors (Temperature & Humidity). The results revealed that mite population (Adult and Nymph) was high in the first week of November with 393 individuals / 10 plants and the mite population (Adult and Nymph) was minimum on the third week *i.e.* 55 individuals / 10 plants. In this regard, RH showed negative relation while the temperature showed positive relation. Thus, the study, will help in better understanding of the population fluctuation (mites).

EXPLOITATION OF HOST PLANT RESISTANCE TRAITS IN COTTON (*GOSSYPIMUM HIRSUTUM* L.) AGAINST SUCKING INSECT PEST COMPLEX

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A host plant resistance (HPR) experiment against sucking insect pest complex comprising of 12 varieties including one glandless variety was conducted. Data on insect infestation and morpho-physiological, biochemical HPR and yield traits were recorded. Potential HPR traits were identified alongwith genotypes possessing better HPR against sucking complex. The genotypes, Bt.30, NIAB-878 and Sadori were found to be high yielding with less attack of sucking insects. It was generally observed that these genotypes possessed medium size life, high leaf glanding, high phenolic and tannin contents, less soluble sugars and proteins as defensive mechanism against sucking insect pest complex. Therefore, HPR is an important tool that can be deployed for enhancing integrated pest management in GM cotton system.

MONITORING AND POPULATION DYNAMICS OF PINK BOLLWORM BY USING PHEROMONE TRAPS IN TANDOJAM, SINDH

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Pink bollworm, *Pectinophora gossypiella*, is a real threat to conventional and Bt varieties of cotton in Sindh. The adult male moth population of pink bollworm was monitored by using specific sex pheromone traps. The study was carried out from the month of January 2016 to December 2017. The trap catches were recorded weekly and abundance of pink bollworm moths in each month was evaluated. The results showed that adult moths were present throughout the year in studied locality, Tandojam, but the numbers seemed variable in different months due to variation in weather condition and availability of host plant. Furthermore, it was observed that population got higher from August to November and reached to their highest peak in September that was the peak season of cotton crop (when cotton crop is fully matured) whereas minimum trap catches was observed in the month of June.

EVALUATION OF BIOPESTICIDAL POTENTIAL OF *METARHIZIUM ANISOPLIAE* TO CONTROL STORED GRAIN PEST *TROGODERMA GRANARIUM*

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As the world population is increasing faster than ever, each year one third of potential food supply is lost to weeds, pest and diseases of crops. *Trogoderma granarium* is one of the most devastating insect pests of stored grain wheat after harvesting. Different control measure including the use of synthetic insecticides and fumigants were used to control this insect pest but it developed resistance against all these control strategies. Keeping in view the problem of resistance development, the current study was aimed to determine the biopesticidal potential of an entomopathogenic fungus *Metarhizium anisopliae* to control this notorious insect pest. The LC₅₀ of *M. anisopliae* was 3.99ppm against 4th instar larvae of *T. granarium*. The toxic effect of sub-lethal dose of *M. anisopliae* to 4th instar larvae was investigated in terms of total protein, soluble protein, total lipids, glucose, glycogen, free amino acid, trehalose, DNA and RNA contents. The effect of sub-lethal dose of *M. anisopliae* on some detoxifying enzymes was also evaluated. After exposure to sublethal dose of *M. anisopliae* the concentration of total protein, soluble protein, total lipids, glycogen, and trehalose were significantly decreased (6.94, 14.8, 22.02, 30.21 and 14.49%)

respectively. The concentration of glucose, free amino acid, RNA and DNA were significantly increased (21.65, 28.81, 3.47 and 6.14%) respectively. The activities of catalase, invertase and trehalase were significantly increased (27.88, 10.54 and 42.66%) but activity of amylase was significantly decreased (48.90%) after exposure of sub-lethal dose of *M. anisopliae*.

SYNERGISTIC TOXICITY OF SPINOSAD AND DELTAMETHRIN AND THEIR TOXIC EFFECT ON METABOLITES OF *TROGODERMA GRANARIUM*

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Trogoderma granarium is one of the most notorious pests of stored grain wheat. To overcome the problem of pest infestation, various insecticides as a single active ingredient have been used but insect pest has developed resistance. So, binary combinations are a new hope to control *T. granarium*. Keeping in view the development of pest resistance, the present study was aimed to determine the synergistic toxicity of spinosad and deltamethrin against 4th instar larvae of *T. granarium*. Their most effective mixture was used to investigate toxic effects on macromolecules/metabolites and few enzymes of 4th instar larvae of *T. granarium*. The concentration of glycogen, glucose and DNA were significantly increased (48.48, 33.33 and 6.52%) respectively after treatment with mixture. The concentration of total proteins, soluble protein, total lipids, free amino acids, RNA and trehalase were significantly decreased (9.06, 20.20, 38.46, 9.19, 8.17 and 14.03%) respectively. The activities of catalase, choline esterase, invertase and trehalase were significantly increased (2.74, 43.28, 9.59 and 47.87%) after exposure to sub-lethal dose of mixture. The activities of amylase were significantly decreased (44.65%) after exposure of sub-lethal dose of deltamethrin and spinosad.

PARASITIZING POTENTIAL OF *DIRHINUS GIFFARDII* (SILVESTRI) AND THEIR POST EMERGENCE SEX RATIO AGAINST VARIOUS AGE PUPAE OF *BACTROCERA ZONATA* UNDER LAB CONDITIONS

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Fruit flies being serious pests of fruits and vegetables are responsible for huge economic losses in the world. Biological control is a sustainable pest management technique which offers naturally sound and effective management of these pests. *Dirhinus giffardii*, pupal parasitoid of fruit fly, is one of the most significant biological control agents which has been effectively used in controlling fruit flies. Laboratory studies were conducted to investigate the preference of *D. giffardii* for different aged pupae of *B. zonata*, at different exposure times (24 h, 48 h & 72 h) for parasitism. Results revealed that mean rate of parasitism was the highest on 3 days old pupae in all the exposure periods of 24, 48 and 72 h of *B. zonata* with maximum parasitism recorded after exposure period of 72 h followed by exposure period of 48 h. In all the exposure periods, the rate of parasitism was in the increasing order from 1-3 days old pupae after which the same was declined significantly with the lowest parasitism rate recorded in 5 days old pupae. In all the different aged parasitized pupae exposed to parasitism for 24, 48 and 72 h period, the post emergence sex ratio of male parasitoids was recorded higher compared to females. The studies manifested that the exposure time had a significant effect on parasitism and that the parasitoids *D. giffardi* can be more efficiently reared on 2-3 days old pupae of *B. zonata* with exposure time of 72 h for parasitization.

EVALUATION OF *NERIUM*, *NERIUM OLEANDER* AGAINST BLACK RAT, *RATTUS RATTUS*

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Many toxic plants can be utilized against rodent pests; however no significant work has been carried out on the subject. In this context, there is a need for identification of natural products which may be useful for rodent management. *Nerium*, *Nerium oleander* (family, *Apocynaceae*; Linnaeus, 1753) is a toxic shrub grown usually as an ornamental plant in tropical and subtropical regions. A study was carried out to know the toxicity of this common plant against the commensal rat, *Rattus rattus*. The plant was tested both in paired-choice as well as in no-choice experiments. Comparison of the palatability of leaf extract added bait with the plain bait also carried out. For the purpose plant added bait was prepared by mixing the leaf extract and wheat flour in three concentrations (20.00%, 10.00% & 5.00%) along with 2% sugar as a taste additive. Acclimatized and individually caged healthy rats of approximately same weight and age were randomly divided into three sets of five rats (mix sexes) for each, to observe the effect of all three concentrations along with one set of control rats (plain bait). Palatability of poison bait in comparison to plain bait (paired-choice) was noted for five days. Time to death and percent mortality was also recorded. Consumption of the bait was inversely proportional to the concentration. *Nerium oleander* gave 69.50% mortality in 3.35 ± 1.62 days at 20.00%, 50.00% in 4.25 ± 2.10 days at 10.00%, 8.38% in 4.00 ± 2.15 days at 5.00% concentrations and 0% in control. The results of the study proved high toxicity and palatability of *Nerium oleander* for *Rattus rattus* ($P < 0.05$) however there is a need for some more studies in this regard.

TOXICITY OF INSECTICIDES AGAINST THE SUCKING PESTS AND THEIR PREDATORY BUGS, ON COTTON CROP

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Field studies on efficacy of different insecticides against the cotton jassid, thrips, whitefly and their toxicity to Heteropteran predator bugs, *Geocoris ochropterus* (Fieber), *Orius* sp. and *Zanchius* sp on cotton CRIS-342 were carried at Entomology Section, Agricultural Research Institute Tandojam during two consecutive years 2011-12. The insecticides tested were imidacloprid, spinosad and indoxacarb and control plot. The pretreatment observation was taken 24 hrs before and post treatment observations were recorded after 24 and 72 hrs 7 days, 10days and 14days after application of insecticides. On overall basis imidacloprid was found to be the most effective insecticides against jassid, whitefly followed by spinosad to thrips. Among the treatments one day after spraying imidacloprid showed superior efficacy in bringing down all the sucking pest population followed by spinosad, at three days after spraying which were found to be superior over other treatments imidacloprid followed by spinosad and indoxacarb. The similar trend was also observed even at seven days after spray. While, spinosad were found comparatively less toxic to predators, *Geocoris* (0.44), *Orius* (0.91) and *Zanchius* (0.80), followed by indoxacarb with of 0.44, 0.90 and 0.75, respectively.

INSECTICIDAL ACTIVITIES OF KITCHEN WASTE BIO-FERTILIZER ON *PHASEOLUS VULGARIS* L. (RED KIDNEY BEANS) PLANT AND COMPARED IT WITH CHEMICAL FERTILIZER

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The aim of this research is to observe the efficacy of bio-fertilizer on the red kidney beans plant and check its insecticidal effects on the plant and also compared it with the plant in which chemical fertilizer was used. By treating

the soil with bio-fertilizer and chemical fertilizer, the insect infestation was observed on them. The study was done in the Zoology lab of Jinnah University for Women, Nazimabad, Karachi from April to September 2018. The major differences were observed in insect infestation between treated and controlled plants. The overall results indicated that the bio-fertilizer that made from kitchen and garden waste efficiently controlled the main insect pests of *Phaseolus vulgaris*, whereas non treated red kidney bean plants showed highly infested growth of mealy bugs. Red kidney bean plants are damaged by several types of insect pests. The major pests are mealy bugs, *Ophiomyia phaseoli* (Tryon), *Aphis fabae* Scopoli and *Oothea bennigseni* Weise which cause the yield loss of about 37% - 100% from *O. phaseoli*, 18% - 31% from *O. bennigseni* and 37% from *A. fabae* (Mwanauta, *et.al.*, 2015). In Pakistan up to 61% of agricultural soil are under major severe threat of deprivation and are strongly scarce in nutrients because of the usage of high concentration of chemical fertilizers and unsuitable agriculture practices. (Sadaf, *et.al.*, 2017).

EVALUATE THE EFFICACY OF ENTOMOPATHOGENIC FUNGUS AGAINST *B. ZONATA* AND *B. DORSALIS* AT DIFFERENT CONCENTRATIONS

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The present study was carried out to evaluate the virulence of *B. Bassiana* against larvae of fruit fly species (*B. zonata* and *B. dorsalis*). Bioassays were carried out under controlled conditions (27 ± 2°C, 80 ± 5% RH, and a photoperiod of 12:12 [L:D] h) Three concentrations [Bb-1 (1×10³, Bb-2 (1×10⁵), Bb-3 (1×10⁷) spores/ml] of *B. bassiana* were tested against *B. zonata* and *B. dorsalis*. It was recorded that Bb-1 (54.54%), Bb-2 (63.25%) and Bb-3 (82.76%) morality against larvae of *B. zonata* after 72h of treatment. However, For the larvae of *B. dorsalis* all tested concentrations were provided significant mortality (49.05%, 59.47% and 93.56%) at [Bb-1 (1×10³, Bb-2 (1×10⁵), Bb-3 (1×10⁷) spores/ml] concentrations. For untreated insects, mortality was lowest 2.27% after treatment of 72h. The fungal mycelium emerged through the soft parts of the exoskeleton, such as the wing bases, mouth, intersegmental regions of the legs, and membranous regions of the abdomen, coxae, and neck. These results indicate the possibility of fruit fly suppression with entomopathogenic fungi.

EVALUATION OF METHOXYFENOZIDE, AN INSECT GROWTH REGULATOR, APPLIED TO DIFFERENT GRAIN COMMODITIES FOR CONTROL OF KHAPRA BEETLE, *TROGODERMA GRANARIUM* (COLEOPTERA: DERMESTIDAE)

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The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is considered as a serious pest of stored products and it feeds on a wide range of dry food products of plant and animal origin, including cereals, museum specimens and dried fish,. The residual efficacy of methoxyfenozide (an insect growth regulator) was assessed by exposing late instar larvae of *T. granarium* to the treated commodities (wheat, maize, rice and oat) at concentrations of 1, 2 and 4 ppm under laboratory conditions. The bioassays were conducted at 0 to 16 weeks post treatment period. Adult emergence was greatly reduced at all the treated commodities at tested concentrations. Adult emergence (at 4 ppm) did not exceeded 28% for the first 9 weeks of the test period; however it reached 80% at 16th week in wheat. Results show that methoxyfenozide can be a potential product for pest management in mills, warehouses and food storage facilities.

BIOCHEMICAL, PHYSIOLOGICAL AND ANTIFUNGAL CHARACTERIZATION OF ACTINOMYCETE ISOLATES FROM *COPTOTERMES HEIMI* GUT**Naveed Ahmad and Ayesha Aihetasham****Department of Zoology, University of the Punjab, Lahore, Pakistan*

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Present study involves the isolation, identification, biochemical and physiological characterization of Actinomycetes from termite (*Coptotermes heimi*) gut and their efficacy against pathogenic fungal strains, which were found positive for oxidase, catalase, and indol acetic acid test. Best growth was obtained at temperatures between 30-37° C and pH (7-9). All of the isolates showed antifungal characteristics against three pathogenic fungal strains *Geotricum candidum*, *Aspergillus flavipus* and *A. asperosems*.

TOXIC EFFECTS OF DIFFERENT PLANT EXTRACTS AGAINST *COPTOTERMES HEIMI***Ayesha Aihetasham* and Ayesha Ayub***Department of Zoology, University of the Punjab, Lahore, Pakistan*

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Coptotermes heimi, a subterranean termite, is an important pest of the Indian subcontinent, causing extensive damage to major agricultural crops and forest plantation. The use of naturally occurring anti-termite compounds, extracted from locally available plants have shown promising approaches towards termite control because they constitute a rich source of bioactive molecules. Present study was carried to study the effect of acetone Leaf extracts of four different medicinal plants: *Nicotiana tabacum*, *Pennisetum purpureum*, *Tagetes erecta*, and *Polyalthia longifolia* on *Coptotermes heimi*. Extract were prepared using Soxhlet apparatus. Different concentrations i.e. 5%, 3% and 1% were used for termite bioassay. Maximum mortality i.e. 100% was recorded at all concentrations of *N.tabacum* however minimum mortality i.e. 49% was recorded at 1% concentration of *P. longifolia*. LT₅₀ of these phytochemicals were also determined which showed *N. tabacum* highly effective against *C. heimi*.

EFFICACY OF ETHANOLIC PLANT EXTRACTS OF *ZINGIBER OFFICINALE*, *RAPHANUS SATIVUS*, *ROSA INDICA* AND *ALOE VERA* AGAINST *HETEROTERMES INDICOLA***Qurat-ul-Ain and Ayesha Aihetasham****Department of Zoology, University of the Punjab, Lahore*

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Present study was performed to evaluate the anti-termite potential of four common plant species of *Zingiber officinale*, *Raphanus sativus*, *Rosa indica* and *Aloe vera*. During laboratory bioassay, the biological activity of ethanolic extracts of these plants was evaluated. The ethanol extract of *Z. officinale* caused highest mortality (100%) while least mortality (68%) was observed in *R. indica* extract. Present findings suggested that these plant extracts can provide environmental friendly management of *H. indicola*.

SECTION – III

ENTOMOLOGY

DIVERSITY OF PHYTOPHAGOUS SCARAB BEETLES (COLEOPTERA: SCARABAEIDAE: MELOLONTHINAE) IN DISTRICT HYDERABAD, SINDH

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Phytophagous scarab beetles refer to order coleoptera, family scarabaeidae and sub-family melolonthinae. Adults as well as larvae are phytophagous and also called chafers. Adults range upto 3-58 mm in size having black or reddish brown color. Head without horns equipped with well developed mandibles. Adults and larvae are significantly important because of their destruction to variety of crops, pastures and grassy lands. Majority of them are nocturnal and crepuscular. Fortnightly observations were made and the specimens were caught by multiple methods (mercury light trap, pitfall trap and hand picking) for six months starting May 2017 to October 2017. Total 1625 specimens were collected and identified into three species of genus *Melolontha*.

ECOLOGICAL ASSESSMENT OF NARMADA RIVER WITH SPECIAL REFERENCE TO DIVERSITY OF INSECTS

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The molluscs 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 2014- March 2017 in selected stations of Narmada river. In present study various species of molluscs 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 through out the study period. Highest Shannon and Weaver index was observed in station I and lowest in station II. The value of correlation coefficient indicates that there was a moderate positive correlation between the pelecypods and transparency, alkalinity, while the gastropods show significant positive correlation with transparency. A moderate negative correlation was between molluscan diversity and BOD, pH, temperature. 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.

FIRST RECORD OF *ZICRONA CAERULEA* (LINNAEUS 1758) (HEMIPTERA: PENTATOMIDAE: ASOPINAE) FROM KHAIRPUR DISTRICT SINDH, PAKISTAN.

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Zicrona caerulea (Linnaeus 1758) (Hemiptera: Pentatomidae: Asopinae) is most important carnivore stink bug produced unpleasant smell scattered in all geographical regions, they are predator on various larva's of Hymenoptera, Coleoptera and Lepidoptera, *Zicrona caerulea* (Linnaeus 1758) has been first time reported from the different

localities of Karachi in Sind, Murree in Punjab, Abbotabad, Balakot, Ghari, Habibullah, Kagan, Naran, Kalam in KPK, Muzaffarabad, Chunari in Kashmir and Sylhet in East Bengal (Nazeer Ahmad Rana 1985), identified on the basis of external morphology as well as internal anatomy dissections female and male genitalia (spermatheca, spermathecal bulb, aedeagus, pygophore and paramers), draw the diagrams *Zicrona caerulea* (Linnaeus 1758) first time reported from the Khairpur district of Sindh Province Pakistan.

BIODIVERSITY OF ANTS (HYMENOPTERA: FORMICIDAE) IN MANGO TREES OF MIRPURKHAS, SINDH, PAKISTAN

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Sindh province is located in southeast of Pakistan, its 65-70% peoples depends on agriculture, it occupied 140,914 km² land. This land produces diverse floral life (cereals, folders, fibers, timbers, sugarcane, fruits crops etc). After banana the second largest growing fruit is mango. It cultivated on 96.42 thousand hectares via yielding 920.7 thousand tones annually. The term biodiversity is variety of life; here it means the association of ants and mango trees. Links of fauna is vital for agriculture like ants because they are Social, Predators, Scavengers, Pollinators, Soil turners, Pests. This study was planed due to ant-plant mutualisms. Collections and observations were carried out in district Mirpurkhas while using bait like chicken visceral, sweets and digging the soil (habitat). Total 250 specimens of different castes were collected and sorted out into three species and genrea. Identifications and classifications were based on the keys given by Bolton, 1994 and Sheela, 2008. This is the first reported research work from Sindh, Pakistan. Pictures were captured by digital Microscope, LED stereoscopic microscope and Digital camera.

DIVERSITY AND ECOLOGY OF ANTS (FORMICIDAE) FROM NAUSHAHRO FEROZE, SINDH, PAKISTAN

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Diversity (variety) of ant's fauna occurring in an ecosystem has diverse and versatile importance. Because of their huge number and functions like soil fertility, predacious, scavenger, pollinators and pest exterminators. They are social insects living in colonies having different cast i.e workers, drone, queen and soldiers. This study was carried out from district Naushahro Feroze (located 26° 50'24" N 68° 07' 12" E) with altitude 38 meters. Total 830 specimens were collected and preserved into 75% ethanol with few drops of glycerin. Collections were made by hand picking and using bait (sweets and chicken visceral) and arranged into fifteen species and five genera. Identification was prepared using keys given by Bolton, 1994; Sheela, 2008; Naumann, 1993 and McArthur, 2001. Pictures were captured by Stereoscopic microscope and digital Camera.

PRAYING MANTISES (DICTYOPTERA, MANTODEA) OF DISTRICT NAUSHAHRO FEROZE

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Praying mantises belongs to Superfamily Mantodea are predators, feeding mainly on variety of insects. Being predatory in nature they are very important for studies concerning with the biological control. During the present study an extensive surveys were carried out in various talukas of district Naushahro Feroze and about 186 specimens

have been collected and identified into 02 Families viz: Mantidae and Toxoderidae with 06 genera and 08 species. Beside this, description for families, genera and species along with digital images were also provided for easily identification of species. Further, highest number of specimens were collected from Taluka Mehrabpur (31.18%) followed by Kandiaro (23.65%) and Bhiria (20.96%) while lowest population of specimens were collected from Moro (11.29%) followed by Taluka Naushahro Feroze (12.90%) respectively. Present study is an initiative step towards the biodiversity of Mantodea fauna of district Naushahro Feroze.

SYSTEMATIC STUDY ON GENUS *HOLOCHLORA* STAL, 1873 (PHANEROPTERINAE) FROM PAKISTAN

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Pakistan is diversified region, having varieties of biogeographical and ecological zones that include: Alpine; sub-Alpine; tropical; sub-tropical; semi-desert; desert saline to down to ocean. The Ensifera fauna of Pakistan is rich, varied and well-marked. This is classified into two main groups 1. Caelifera (Commonly known short-horned) 2. Ensifera (Mostly termed long-horned). Species belonging to genus *Holochlora* Stal 1873 are generally phytophagous and are widely distributed throughout the country due its different geographical zones. During the current study nearly 260 specimens of this genus were collected and identified into five species i-e: *Holochlora japonica*, Brunner von Wattenwyl, 1878, *Holochlora nigrotympana* Ingrisch, 1990, *Holochlora venosa* Stål, 1873, *Holochlora astylata* Karny, 1926 and *Holochlora nigropinulosa* Brunner von Wattenwyl, 1893. *Holochlora nigropinulosa* Brunner von Wattenwyl, 1893 constructed new record for Pakistan while other species were redecribed from study are. In addition to description of species, taxonomic keys for the separation species and distributional data is also provided. Additionally, the necessary illustrations and digital photographs are also given.

FAUNISTIC STUDY ON THE DRAGONFLIES (ODONATA) FROM DISTRICT LARKANA, SINDH PAKISTAN

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Dragonflies (Odonata) are well known bio-control agents and environmental indicators that are commonly found in running as well as standing freshwater bodies. They possess slender abdomen, large eyes, short antennae and long wings. They are important predators of serious insect pests of crops, fruits and vegetables. Their larvae are also voracious predators and consume mosquito larvae. An extensive field surveys were conducted to collect Dragonflies fauna of Larkana district, during March 2018 to September 2018, in the different sites of the study area. A total of 215 specimens were captured and identified into 09 species in 05 genera, belonging to two families. Family Libellulidae having 08 species belonging to 4 genera, while only one species belong to Family Aeshnidae. The family Libellulidae includes the major numbers of species as compare to Aeshnidae. The species were, *Orthetrum chrysis*, *Orthetrum sabina*, *Orthetrum pruinosum neglectum*, *Orthetrum cancellatum*, *Acisoma panorpoides*, *Pantla flevescens*, *Crocothemis nigrifrons*, *Crocothemis servilia*, and *Anax imperator*. Beside this, identification keys were also provided for easily isolation of families and genera. The surveyed area showed diverse dragonfly fauna and thus further extensive surveys are recommended that can come up with more important species from the area.

BIODIVERSITY OF BEETLES (ORDER COLEOPTERA FROM DISTRICT MIRPURKHAS SINDH, PAKISTAN

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Present study was conducted during the year of 2017 was exploration biodiversity. A total of 200 specimens, 3 Families (Carabidae, Scarabidae, Cerambycidae), 4 genera (Anthia, Melolontha, Aneflus, Carabus), 6 species (*Anthia sexguttata* (Fabricius 1775), *Melolontha haptica* (Fabricius, 1801), *Melolontha pictorica* (Germer, 1824), *Carabus hortensis* (Linnaeus, 1758), *Aneflus calvatus* (Horn 1885), collected from the different areas of district Mirpurkhas Sindh Pakistan.

MORPHOMETRIC CHARACTERISTICS OF DRAGONFLIES AND DAMSELFLIES FROM DISTRICT MATIARI

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During the present field survey, we observed the different species of Odonata. A total of 350 specimens were collected from the different areas of District Matiari. Material sorted out into 6 species *Orthetrum chrysis* (Selys, 1891), *Orthetrum Sabina* (Drury, 1770), *Bradinopyga geminata* (Rambur, 1842), *Ischnura elegans* (Vander Linden, 1820), *Ischnura verticalis* (Say, 1839), *Ischnura ramburii* (Selys, 1850) belonging to 3 genera (Orthetrum, Bradinopyga, Ischnura) 2 families (Libellulidae, Coenagrionidae).

PREVALENCE OF CHEWING LICE (PHTHIRAPTERA: INSECTA) ON DIFFERENT BREEDS OF DOMESTIC FOWLS (AVES: GALLIFORMES) FROM DISTRICT HYDERABAD, SINDH, PAKISTAN

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The Chewing lice (Insecta: Phthiraptera) are small tiny wingless common ectoparasites of avian fauna. The lice cause parasitism and have high capability to develop host specificity. They cause infestation in hosts by reducing their health, feathers, eggs and meat production. Poultry birds or chicken are big source of nutrition to man. Presently, three breeds of one type of medium size galliform bird, domestic fowl *Gallus gallus* was selected for collection, identification, population density and rate of infestation of chewing lice from different regions of District Hyderabad, Sindh, Pakistan. These breeds of chicken are Aseel chicken, Sonali chicken and Misri chicken. 47 Domestic fowls *Gallus gallus* were collected and brought into the laboratory. The study was conducted from April 2016 – March 2017. Domestic Fowls were kept on white paper sheet for about 30 minutes and sprayed with Permethrin powder in their wings. The infested birds were tagged with identity rings to check their lice after interval of 2 to 3 weeks. The chewing lice species were collected and preserved in 70% ethanol. The permanent microscopic slides were prepared with Canada balsam through standard method of preservation. Currently 225 chewing lice were recovered belonging to 03 genera and 05 species. These species and their Prevalence are 27.55% for *Menopon gallinae* (Linnaeus, 1758) 25.77% for *Goniocotes gallinae* (de Geer, 1776) 19.11% for *Goniodes dissimilis* Denny 1842 for 12.52% *Menacanthus stramineus* (Nitzsch, 1818), and 11.11% for *Lipeurus tropicalis* Peters, 1931. All these 05 species were reported first time as new host record and new locality record from the study area. All the chewing lice species were described in detail, compared with related species and a taxonomic key has been constructed.

EXPLORING THE DIVERSITY OF GRASSHOPPER (ORTHOPTERA) FAUNA IN DISTRICT MARDAN KP, PAKISTAN

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The current study was conducted from August 2016 to August 2017 on the diversity of grasshopper fauna of District Mardan KP Pakistan. During the study 17 different species belonging to 17 genera 4 families, 1 order (Orthoptera). The reported species are, *Chorthippus albomarginatus*, *Chrysochraon brachypterus*, *Bermius sp.*, *Chortophagga viridifaciata*, *Chrysochraon dispar*, *Leptysma marginicollis*, *Phlaeoba infumata*, *Pseudopomala brachyptera*, *Chorthippus brunneus*, *Arphia simplex*, *Chorthippus parallelus*, *Omocestus viridulus*, *Poekilocerus pictus*, *Atractomorpha similis*, *Paratettix cucullatus*, *Paratettix aztecus*, *Tettigoniida scudderii*. During the study the family Acrididae was the most common family of the collection points at both planed and hilly areas, contain 12 species out of 17 reported species, while the family Tettigoniidae was the rarest family of the area which only contains 1 species out of 17 reported species.

TAXONOMIC SURVEY OF ORB WEB SPIDER (ARACHEAE: ARACHNIDA) OF WHEAT CROP FROM DISTRICT, DADU, SINDH, PAKISTAN

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In Sindh 3.5 million tons wheat is produced and Ranked at second number in Pakistan. Wheat is infested variety of pests like aphids, caterpillars, crickets, wireworms, leaf beetles, grasshoppers etc. Farmers mostly use pesticides in order to save their crops but it creates enormous dangers to environment. Using of biological control method is basic safest, self established, reducing the rate of pests' population within effective IPM. Arachnida has group of spiders on the 7th number in biodiversity More than 47000 identified species with 114 families are known worldwide. Spiders are biological control agents, voracious, carnivorous and generalist predators. During the present study, wheat field of District Dadu was surveyed in the month of December 2017 to April 2018. 527 specimens were collected and sorted out into two families namely Araneidae, Tetragnathidae. All families were classified up to Genera and species level. *A. trifasciata*, *A. Pradhani* of family Araneidae, *Tetragnathajavana* of family Tetragnathidae are first time recorded from this area. Identification of given species of different families up to generic level as well as species level with the help of taxonomical keys.

SEASONAL VARIATION IN SPECIES COMPOSITION OF APHIDOPHAGOUS HOVERFLIES IN AGRO- ECOSYSTEMS OF HYDERABAD REGION

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The Syrphid flies of subfamily Syrphinae are very essential group of insects having banded and striped abdomen. These beautiful flies resemble with small bees, often baptized flower flies or syrphid flies. The Syrphinae flies are diurnal flies having small to large size with worldwide dispersal but customarily favor to conscious in comparatively cold weather conditions generally set up near the blossoming plants. The immature stages of these Syrphinae flies are important due to their predatory habit, most of these are aphidophagous in nature feeding on all kinds of aphid species, whereas the adults of these flies are measured

advantageous insect, play significant role in cross-pollination of various agricultural and non-agricultural crops. The varieties of aphidophagous syrphid flies were captured from various agro ecosystems of four districts of Hyderabad region, Sindh i.e. Tando Jam, Jamshoro, Tando Muhammad Khan and Matari on different host plants. These beautiful hoverflies were arbitrarily collected using insect hand net then mounted with standard method. Seasonal variation and population dynamics of aphidophagous hoverflies (Syrphidae: Syrphinae) in relation to abiotic as well as biotic factors were studied during January to December 2018 in Hyderabad region Sindh Pakistan. The community of Syrphinae hoverflies was composed of 920 individuals belonging to 4 species which were recorded from 25 plant species. Among these species *Episyrphus balteatus* (530) was the most abundant species visiting 18 host plants whereas *Sphaerophoria riarupelli* (40) was the least abundant species visiting 7 host plants only. The peak abundance and richness of hoverflies was observed in spring (March-April), the time when the maximum number (20) plant species were at flowering stage in studied localities. The results showed that *Episyrphus balteatus* remained active throughout the year in variable abundance. Among agricultural and wild plant species, *Mangifera indica* and *Launaeapro cumbens* were visited by the maximum number of syrphinae species. Abundance of hoverflies was positively correlated with the floral abundance and flowering plant species, while relative humidity, temperature and rain fall have negative or very weak correlation. The results of this study revealed that the abundance of flowering plant species and aphid species were positively correlated with the population of syrphinae hoverflies, even the thickness of aphid colony has strong correlation with Syrphinae population while relative humidity, temperature and rain fall have negative or very weak correlation.

BIODIVERSITY OF NOCTUIDAE (LEPIDOPTERA) FROM DISTRICT KHAIRPUR MIRS SINDH PAKISTAN

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The moths belonging to family Noctuidae are voracious insects and consume wide variety of agricultural crops. During the present study a total of 150 specimens were captured and were sorted out into 10 genera and 15 species i.e. *Grammodes stolidus* (Fabricius, 1775), *Spodoptera litura*, (Fabricius, 1775), *Meterana pansicolor* (Howes, 1912), *Helicoverpa armigera* (Hubner, 1808), *Helicoverpa zea* (Boddie, 1850), *Agrotis ipsilon* (Hufnagel, 1766), *Mythinma unipuncta* (Haworth, 1809), *Meterana alcyone* (Hudson, 1898), *Heliothis peligera* (Denis & Schiffemuller, 1775), *Heliothis adacta* (Butler 1878), *Mythinma impurea* (Hubner, 1808), *Dipaustica epiastrea* (Meyrick, 1911), *Graphania pagaia* (Hudson, 1909), *Agrotis exclamtionis* (Linnaeus, 1758), *Dysgonia algira* (Linnaeus, 1767). Beside this the distributional data along with description of species and taxonomic keys are also provided.

DISTRIBUTION AND INCIDENCE OF GENUS *AILOPUS* (OEDIPODINAE: ACRIDIAE: ORTHOPTERA) FROM DISTRICT DADU

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The Genus *Aiolopus thalassinus thalassinus* (Fabricius 1781) represents one of the most important member of the order orthoptera, *Aiolopus thalassinus thalassinus* is reported as a Major pest of Rice, Sugar cane, wheat, Maize & Grasses during the Present study. Its important life parameters i.e. Mating, Copulation, Oviposition and

Development of different instars were studied. A total 1240 specimens including both mature and immature were collected from different localities of district Dadu such as, village Sita, village Makhdoom Bilawal, village Syed Naban Shah, village Hafiz Meer Muhammad Kalhoro, village Ghulam Hussain Gadhi, village Muhammad BachalBouk and village Loung Khan Lund of District Dadu, during the year 2015-2016. *A. thalassinus thalassinus* is a major pest in the District Dadu. This species consume wide variety of food plants Jowar, (*Sorghumbiclor*), Maize (*Zea mays*), Grass (*Cynodondactlon*), Rice paddy (*Orziasavia*) and other cearels. Laboratory studies were conducted on the biology of *Aiolopus thalassinus thalassinus* (Fabricuis1781) including egg laying Behavior, Matting Behavior, life span, Number and structure of eggs and egg pods, and oviposition. It was observed that six nymphal instars of *A. thalassinus thalassinus*. Females lay their egg pods in moist soil by forming a small hole. Pre copulation and pre oviposition period was 05 and 14 days respectively and inter-oviposition period was about 3-6 days. A female laid averagely 04 egg pods and containing about 05-20 eggs in each egg pod. Egg size of *A. thalassinus thalassinus* length (3.5-4.00) width (4.00 – 4.5) and size of egg pod Length (9.5- 12.00) width (3.00- 4.5) Total longevity time period from first nymphal instar to adult was 30 days. and average adult longevity time period was 52 days.

DIVERSITY AND GEO -REFERENCING OF ORTHOPTERA (INSECT) FAUNA OF THAR PAKISTAN

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Thar Desert of Sindh harbors a very rich and highly specialized fauna of insects, but only a few species are still known from Thar. At present 03 extensive surveys were carried out in different habitats in Thar that include: Sand hills, stony rigid hills, clay or salt plains and other typical desert formation during the year 2018. Presently, fair numbers of 03 super families i.e. Tegtigonidea, Schizodactylidae and Grylloidea pertaining to 29 species of various taxa were captured. Their taxonomic revision and biogeographically description was provided. Ecology and bioacoustics was investigated in field. Beside this, its geo-referenced was also given by using Google Earth Pro: 7.3.0.3832 (32-bit) aiming to provide a solid basis for understanding the distribution pattern of the Orthoptera in world. This study is funded by Higher Education Commission, Islamabad, under research project No. 6737 SINDH\NRPUR&D \ HEC \ 2015.

STUDY OF LIFE-FORMS IN ACRIDIDAE (ORTHOPTERA) OF HYDERABAD SINDH, PAKISTAN

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During present work 11 species and subspecies of the tribe Acrotlyini and Locustini were studied and its different life forms were observed. It was noticed that *Acrotylus humbertianus* *A. longipes subfasciatus* *A. longipes subfasciatus* are Terri-arenicole, *A. insubricus insubricus*, *A. patruelis* are Terri-deserticole. *Herrich-Schaffer* and species of tribe Locustini namely, *Locusta migratoria* are Graminicole, Therefore, an attempt has been made to study the life forms of acrididae of Hyderabad and preliminary result is presented here. The occurrence of various life forms of grasshoppers indicate their favorable environmental conditions. Beside this, the insect distribution were also observed.

ELEMENTAL ANALYSIS OF PREFERRED FOOD PLANTS OF OEDIPODINAE (ORTHOPTERA) UNDER SCANNING ELECTRON MICROSCOPE (SEM)

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Oedipodinae is considered the major and minor pest of many valued crops. Presently in order to know its likeness and dis-likeness some observation of food plants were done under Scanning Electron Microscope (SEM). species of Oedipodinae i.e *Gastrimargus africanus sulphureus* Bei-Beinko, *Oedaleus roscens* Uvarov, *O. senglensis* Krauss, *Scinththaristia notabilis cincitipes* Uvarov, *S. notabilis pallipoes* Uvarov, *notabilis brunneri* Uvarov, *Mioscirtus wagneri rogenhoferi* Saussure, *Sphingnotus savignyi* Saussure, *S.sindhensis* Bughio *et al.*, *S. abkari* Wagan & Baloch, *S.rubescens rubescens* Walker were reared on selected food plants i.e *Oryza sativa* L.(Rice) *Pennisetum glaucum* (Pearl Millet), *Piper betel* (Pan), *Mentha viridis* (Mint), , *Lagenaria siceraria* (Bottle gourd), *Momordica charanta* (Bitter gourd or Karela), *Cyamopsis tetragonoloba* (Cluster been), *Saccharum officinarum* (Sugar cane), *Capsicum annum* L., (Chili), *Coriandrum sativum* (Coriander), *Solanum melongena* (Brinjal), *Brassica oleracea* (Cabbage) were scanned under scanning electron microscope (SEM) through ESM (Energy Dispersive X-Ray Spectrometer and spectrum acquisition) it was found that rice leaves possess highest normal weightage percentage of oxygen i.e. 74.14% followed by carbon i.e 14.75% opposing to this minimum percentage was obtained for Phosphorus , Magnesium, Aluminum, Potassium, Chlorine and Silicon respectively. Present study recommends that higher and lower percentage of elements shows the preference of insect on selected plant.

DIVERSITY OF PYRGOMORPHIDAE (PYRGOMORPHIDAE: ORTHOPTERA) SPECIES FROM SUKKUR DIVISION, SINDH, PAKISTAN

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During the year of 2017-2018 extensive survey has been made to collect the information about the diversity of family pyrgomorphidae from various areas of Sukkur division. During the collection it was noticed that some species of genus *Chrotogonus*; *Chrotogonus trachypteru strachypteru* and *Chrotogonus trachypteru robertsi* found in sandy areas of Rasoolabad and Halani. Lot of specimens of genus *Pyrgompha*; *Pyrgompha bispinosa bispinosa* and *Pyrgompha bispinosa deserti* were collected from the rocky areas found in the sides of Theri, Kotdiji and Mehranu. Cultivated areas of Sukkur like Saleh pat, kumb and Sides of Ranipur offered mixed species of Pyrgomorphidae i-e *Atractomorpha acutipenis blanchardi*, *Poeciloceru piuctus*, *Chrotogonus trachypteru trachypteru* and *Pyrgompha bispinosa bispinosa*.

INCIDENCE OF ENSIFERA (ORTHOPTERA) FROM KHAIRPUR MIRS SINDH

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The Ensifera (long-horned) occurs in wide varieties of habitats i-e trees, shrubs, herbs and grasses, vegetation, forest and swamps. They cause considerable damage to all but particularly fruits were more effective by this. In result of extensive survey a total of 382 specimens were come in collection which was sorted out into 03 super families i.e. Tegtigonidea, Schizodactylidae and Grylloidea pertaining to 12 species. It was observed that, the most dominant subfamily was noted Gryllinae with (48.17%) followed by Phaneropterinae with (20.41%) and least common was Conocephalinae with (11.51%). Beside this, highest ratio of *Acheta domestica* with 17.81%, followed by

Trigonocorpha unicolor with 14.13% was observed. However, detail identification keys, based on easily recognizable morphological characters was also provided this work will be very helpful for the control planning in near future.

INCIDENCE OF *HIEROGLYPHUS ORYZIVOURS* (HEMIACRIDIDAE: ACRIDIDAE: ORTHOPTERA) ON DIFFERENT RICE VARIETIES

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The present survey was carried out to know the incidence of *H. oryzivorous* on different rice varieties in 02 different forms of *H. oryzivorus* (Brachyterous and Macropterous form) which are the vigorous pest of the paddy crops in Sindh. The investigation was carried out during the month of the June 2016 to November 2017. Total of 1575 specimens were collected from Buxapur and Badani from different localities of Kashmore district. The incidence was highest in rice fields particularly Dhaga (Hybrid rice variety) 28.6% in 2016 while it was 38.9.0% during the 2017. IRRI NO 6 was 8.52 % in the year of 2016 while it was 8.38% during the year of 2017. The infestation of the Pukhraj (hybrid rice variety) was 3.17% in the year of 2016 while 3.61% was in the year of 2017. The incidence of this pest was 4.57% in 2016 while 4.38% were recorded in Royal (Hybridrice).

SOME OBSERVATIONS ON CHEMICAL DEFENSIVE BEHAVIOR IN ORTHOPTERAN'S SPECIES

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Members of genus *Acrotylus* are major pest of arid and semi arid areas they also demonstrate wide diversity in their food preference. Nymphal stages of its two dominant species i.e *A. humbertinus* and *A. longipes subfasciatus* were studied in detail during the year 2014-2016. Nymphal stages were mostly herbivorous and grammivorous in nature and occupied different ecological niche. First of all food preference of various hopper stages of these species were investigated through regular field surveys and selected a set of five favorite food plants for laboratory rearing. It was concluded that total nymphal duration of *A. humbertinus* was significantly shortest on *Sorghum bicolor* with an average period of 25.56 ± 0.26 and 30.90 ± 0.56 days in male and female respectively whereas, prolonged duration was noted on *Abelmoschus esculentum* with an average of 38.28 ± 0.50 and 43.67 ± 0.29 days in male and female. In contrasting to this, total nymphal period of *A. longipes subfasciatus* was significantly fastest on mixed diet i.e 30.16 ± 0.57 and 30.24 ± 0.21 days in male and female while, *Saccharum bengalense* remained unsuitable food plant and prolonged the nymphal duration up to 45.62 ± 0.59 and 38.92 ± 0.52 days in male and female respectively. However, survivability of hopper stages was maximum on mixed diet; this knowledge will provide basic guideline to identify which plant is preferable by pest.

STUDY ON ENVIRONMENTAL ADAPTATIONS OF PYRGOMORPHIDAE (ORTHOPTERA) WITH SPECIAL REFERENCE TO ITS HOST PLANTS VARIATIONS

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In order to know the Environmental adaptations and host plants variations of two genera *Poekilocerus* and *Chrotogonus* extensive field surveys of different habitats were carried out. Collected samples were sorted out into

Poeciloceris pictus (Fabricius, 1775) and *Chrotogonus* i-e *Chrotogonus* (*Chrotogonus*) *homalodemus* *homalodemus* (Blanchard, 1836), and *Chrotogonus* (*Chrotogonus*) *homalodemus* (Blanchard, 1836). It was noticed that large numbers of *Chrotogonus* were found on open surfaces with scarce number on *Cynodon dactylon* (Grasses), *Hordeum vulgare* (Barley), *Pennisetum glaucum* (Bajra), (Wheat) *Gossypium* (Cotton) and *Sorghum vulgare* (Jowar) and where as *Poeciloceris* was found on Akk plants with scarce on *Citrullus vulgaris* (water melon), *Saccharum bengalense* (sugarcane), and *Medicago sativa* (lucerne). During the field survey it was noted that *Chrotogonus* has very strong adaptations with the environment as compare to *Poeciloceris*. They can tolerate the harsh conditions and survive for longer period of time. Present research suggests that prolong survival of *Chrotogonus* causes great economic losses in region. Beside this, its exact identification along with environmental adaptation on the molecular bases is in progress. This research financially supported by Higher Education Commission, Islamabad, Pakistan (Project No. 6737 SINDH /NRPU /R&D/ HEC/ 2015).

INFESTATION OF GRYLLIDAE (ORTHOPTERA) IN RICE FIELD FROM LARKANA, SINDH

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Rice is the largest used production in our country. The land for producing food variety of rice cultivation present in Sindh. The 03 species of Gryllidae identified from District Larkana. These include reported species are harmful to rice cultivation and also contaminant to ecosystem. *Acheta chudeani* (Chopard, 1963), *Acheta meridionalis* (Uvarov, 1921) and *Gryllus bimaculatus* (De geer, 1773). These 03 species of Gryllidae decrease rice crop production and also spread human diseases because they are parasitized by ticks and mites etc. Present study will be effective for the control of these pest.

DISCOVERY OF *MICROTHESPIA SINDHENSIS* (MANTODEA: DICTYOPTERA) FROM DISTRICT DADU, SINDH, PAKISTAN

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Order Mantodea is the faction of eye-catching, valued, voracious insects of Praying Mantids which are unanimously identified as ensnaring fast predators. During extensive studied exploration of Mantids biodiversity from Dadu region species of genus *Microthespis* was collected during the studied years' i-e: 2016-2018. Dadu have been the remarkable region regarding the records of Mantids species i-e *Rivetina rasheedi*, *Mantis religiosa akbari* and *Microthespis sindhensis*. Morphological characters of collected species advocated a close resemblance with *M. sindhensis* which was documented by Soomro *et al.*, (2001) from this region. In the rest of extensive survey *Microthespis sindhensis* was come in collection after gap of 18 years. Collection of this species from this region shows its rare status in Sindh. This species is very closely resemble with *Microthespis dmitrievae* Werner and *Microthespis evansi* Uvarov except the prosternum with the heart shaped black blotch as in *Microthespis dmitrievae* prosternum with the black transverse blotch on anterior side and large black spot on the posterior side. In *Microthespis evansi* prosternum without any black blotch or spot. Both species *Microthespis dmitrievae* and *Microthespis evansi* first reported by Naheed *et. al* in 2001 from District Dadu, now I have reported only single species *Microthespis sindhensis* after long time from its recorded region Dadu. The present research study re-portrayed this obvious remarkable species of mantids after the gap of period. The taxonomic studies were carried out and compared with pioneered renowned species from Sindh, Pakistan. The diversity of this species was under appreciation because single species was recorded during research study in the various ecological zones from Dadu.

INFESTATION OF *GRYLLOTALPA* (GRYLLOTALPIDAE: ORTHOPTERA) IN SEEDLING CROPS FROM KHAIRPUR MIR'S, SINDH, PAKISTAN

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The members of the genus *Gryllotalpa* all are burrowing insects living deep in dumpy and moist soil near the crops like rice, wheat, cotton and sugarcane and they are also found near the bananas. Their burrowing activity is very harmful for the crops in early stage due to their burrowing activity the roots of crops are damaged and can cause great damage to the fields. Due to their burrowing activity the pastures are also harmed along with this they also cause great damage to pastures in the golf grounds.

OCCURENCE OF DARKLING BEETLES (TENEBRIONIDAE: COLEOPTERA) FROM LOWER SINDH

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During the present study 100 specimens of Darkling beetles were collected from lower Sindh during March 2017 to August 2018. Total 4 localities i-e Tandojam, Jamshoro, Hyderabad, Mirpurkhas were surveyed. Collected specimens were sorted out into 3 subfamilies viz: Stenochiinae, Pimeliinae and Tenebrioninae and 04 species i.e. *Promethis semisulcata* (Fairmaire, 1882), *Trachyderma philistinia* (Reiche & saulcy, 1857), *Gonocephalum hispidocostatum* (Fairmaire, 1883) and *Uroma excise* (Gebien, 1913) were identified. During the present investigation significant morphological characters along with identification keys, photographs and illustrations were highlighted. Further, it was also noted that samples of *Trachyderma philistinia* were highest in number while it was first time recorded in different localities of lower Sindh. The occurrence of many previous recorded species also extent and this new habitat was found.

INVESTIGATION ON THE FAUNA OF ACRIDIDAE (CAELIFERA: ORTHOPTERA) FROM THAR DESERT SINDH

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During the present study an attempt has been made to collect the grasshoppers' fauna from Thar Desert. At the present 173 specimens were collected and sorted out into 2 subfamilies i.e Spathosterninae and Calliptaminae pertaining to 2 species viz: *Spathosternum prasiniferum* (Walker, 1871) and *Acorypha glaucopsis* (Walker, 1870). Further, sampling was done from 02 districts, Tharparkar and Umerkot. In addition to collection, taxonomic key along with the complete taxonomic account for each species was also given for easy recognition of taxa. Capturing of huge material from this region is significant addition in the wealth of Thar Desert. This study financially supported by Research Project No. 6737 SINDH/NRPU/R&D/HEC/2015.

AGGRESSIVE BEHAVIOR IN FIELD CRICKETS (GRYLLIDAE: ORTHOPTERA) FROM DISTRICT NAUSHAHRO FEROZE, SINDH, PAKISTAN

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Naushahro Feroze occupies different types of crops are found in Naushahro Feroze i.e. rice, sugarcane, wheat, cotton, maize, vegetables and fruits. Naushahro Feroze is the cultivated land and there is large number of Field

Crickets cause economic loss. They are nocturnal and produce sound through stridulation. The Field Crickets are found in a broad variety of habitats i.e. trees, shrubs, herbs and grasses. These are the pest of several crops i.e. bajra, maize, wheat, sugarcane and cotton. During field survey it was observed that Field male crickets show dominant, aggressive behavior for two reasons firstly when they copulate with females and secondly to protect their territory. Usually 06 Aggression Levels are found in Crickets i.e. Level 1; Pre-established dominance, Level 2 Antennal fencing, Level 3 & 4 Uni- & bi-lateral mandible spreading, Level 5 mandible engagement and Level 6 Wrestling (all-out fight). Detail study on the aggressive behavior is still in progress.

SYSTEMATIC STUDY ON THE OF CICADA (HEMIPTERA) FROM UPPER SINDH

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Cicada is easily recognized by their loud noise. Found mostly in tropical areas. At present various extensive surveys were carried out from upper Sindh. A single species *Platypleura octo guttata* (Fabricious 1798) was collected. At 3:30 O'clock, which is very preferable time for its collection in Jamshoro. This insect also sings at this time due to its singing we were attracted towards this.

INVESTIGATION ON THE OVIPOSITION SITES OF ACRIDIDAE SPECIES FROM DISTRICT SANGHAR

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Sanghar district is one of the largest district of Sindh province, Pakistan. It is very fertile land but attacked by numbers of pest insect into different crops. Acrididae species mostly copulated during the month of late August to Mid-September. After this they dispersed for example in agricultural fields. They are aggregated at these sites their suitable oviposition sites include fields rice, wheat, cotton, millets fields etc. Corner of fields, shadows of trees or shrubs which have ground, covered of fallen leaves or under heaps of stalks along withered vegetation. Mostly egg-pods are found in the cracking areas of the field.

COMPARATIVE STUDY ON THE LIFE HISTORY STATISTICS OF *OXYA HYL A HYL A* (ACRIDIDAE: ORTHOPTERA) ON DIFFERENT FOOD PLANTS UNDER LABORATORY CONDITIONS

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Comparative study on the life history statistics of *Oxya hyla hyla* Serville, 1831 on different food plants was observed under laboratory conditions. The food preference of insect on different diet showed the life span of *Oxya hyla hyla* after emergence from sixth nymphal instar in female was 32.75 ± 3.30 days while in case of male it was 24.5 ± 2.88 days. Similarly, life history statistics of nymphal developmental duration was noticed in male was 31.6 ± 3.29 days while in female it was 39.6 ± 3.89 days. The growth index for adults on host plants were evaluated in following descending order of *Oryza sativa* > *Zea mays* > *Triticum aestivum* > mixed diet. Comparison among

different food plants showed that adult and nymphs survive for maximum period on *Oryza sativa*, while minimum survival on *Triticum aestivum*. Besides this, hopper also shows significant closer association with their specific habitat compared with adult. It was also noticed that nymphs are often more restricted in their food and habitat while, in some species of Oxyinae there is also change in behavior during entire course of nymphal development, younger are followed more sedentary and tend to be confined to the limits of the original habitat. Besides this, older stages are more mobile and remain tend to disperse anywhere when ever find chance. Feeding behavior also found changed some time they show aggregation behavior in cage, and in high aggregation there was catabolism also noticed.

SYSTEMATIC STUDY OF SUBFAMILY ACRIDINAE (ACRIDIDAE: ORTHOPTERA) FROM DISTRICT MATIARI

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The present attempt was designed to study the systematics of Acridinae grasshoppers. 04 different localities i.e. Matiari, Khybar, Sekhaat and Hala were selected and a total of 370 specimens of subfamily Acridinae grasshoppers were collected. The material was collected and identified from 2017 to 2018 from the varieties of cultivated and uncultivated fields, orchids, thick, long grass land and road side of District Matiari. This subfamily of grasshoppers commonly called silent slant faced grasshoppers. This subfamily grasshoppers commonly called slant faced grasshoppers. Present 04 species namely i.e. *Acrida exaltata*, *Truxalis eximia eximia*, *Phlaeoba tenebrosa* and *Duroniella laticornis* were identified. Their taxonomic status along with various morphometric parameters were analyzed.

OCCURRENCE OF PRAYING MANTIDS (MANTODEA: INSECTA) FROM SANGHAR, SINDH, PAKISTAN

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Praying Mantids play an important role in agriculture sectors protection as predator and biological agent. The taxonomic study of Praying Mantids is neglected and poorly investigated from Sindh, Pakistan therefore this attempt has been made. During the present survey about 31 species and 18 genera of praying mantids comprise on 07 families i.e. Amorphoscelidae, Eremiaphilidae, Empusidae, Hymenopodidae, Liturgusidae, Mantidae and Tarachodidae were collected. Present study recommend that if their culture will done on the commercial level large area of agriculture will be protected. More research on Praying Mantids is needed which will help to understand the current status of Praying Mantids fauna in Pakistan.

STUDY ON THE DEVELOPMENTAL STAGES OF AULACOBOTHRUS LUTEIPES LUTEIPES (ACRIDIDAE: ORTHOPTERA) FROM SINDH

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Survey was carried out a total of 528 specimens of *Aulacobothrus luteipes luteipes* were collected through random visits from various localities of Sindh. A large number of immatures of *Aulacobothrus luteipes luteipes* were collected and sorted out into different stages. This species seems to be one of constant and serious pest of important

crops. For easily identification the measurement of various body parameters, identification key and line drawings were presented. Study of its immatures was useful to take any control measures.

DEFENSE MECHANISM IN BUSH CRICKETS (PHANEROPTERINAE: TETTIGONIIDAE) IN JAMSHORO

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Bush crickets are found in habitat patches of vegetation at the different heights. Bush crickets are mostly found in clusters on the top of different altitudes of vegetation. Female bush crickets spend long time deep inside the thick vegetation where they search for the suitable site for oviposition and at the same time they do not respond to the male call therefore, females are not easily identified by the predators. That's why the female crickets are less damaged than the males by the selective pressure cause by the predators. The bush crickets whose main character is to protect themselves from predators is camouflage, this type of thick vegetation provides them a good shelter to defense from enemies and at the risk of predation they remain motionless. Therefore, the thick vegetation is most favorable for the both adult male and female to vanish deep down to the base of vegetation when their approach reach to the two meters in vegetation. This study is financially supported by Research Project No. 6737 SINDH/ NRPU/R&D/HEC/2015.

PRELIMINARY OBSERVATION ON CAMOUFLAGE BEHAVIOR OF *CARAUSIUS (DIXIPPUS) MOROSUS* (PHASMATODEA: PHASMATIDAE) IN NARA DESERT

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Camouflage is the unique quality of phasmids presently it is well observed in *Carausius morosus* because it appears easily. In order to know the camouflage behaviour of *Carausius* 5 extensive surveys were carried out in the month of September to October in 2017 to 2018. In the result of these surveys 87 specimens of stick insect were collected and wondering only single species i.e. *Carausius (Dixippus) morosus* (de Sinety 1902) was reported which shows its rare status in Pakistan. The change in color depends upon morphological and physiological conditions of the stick insects. It was observed that the change in color is due to change in the atmospheric factors like temperature, humidity and light which influences morphological color to change through the pigment synthesis. There are the different examples of different morphological pigments which synthesized by environmental factors i.e carotenoids, melanins, pterins, ommochromes and tetrapyrrols. During the postembryonic development it is found that the composition of carotenoids become changed. The physiological change occur due to migration of pigment granulae along with microtubule bundles in the epidermal cells and it also caused by environmental factors. However, this study still in progress.

STUDY ON POPULATION DYNAMICS OF APHID AND THEIR NATURAL ENEMIES ON THE DIFFERENT WHEAT VARIETIES IN LARKANA PAKISTAN

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The study on the population dynamics of aphids and their natural enemies on different wheat varieties was conducted during 2017 to determine the population dynamics of aphid on 05 wheat varieties viz: TD1, Banazir, Tj83,

QS4, NIA amber and their natural enemies at Abbasi farm near Larkana. Five sub plots were selected for each wheat variety, 10 plants randomly selected per plot. Aphid infestation started from January and lowest percentage of infestation was observe di-e(0.3) but reached at peak i-e (47.6) in the mid of March. Maximum No. of aphids and natural enemies were counted on wheat variety TD1 and minimum No. of aphids counted on NIA amber. Natural enemies such as, lady beetle, syrphid fly, green lace wing, aphid midge, spiders etc were found and counted. It was observed during the survey that the population of lady beetles was highest then other natural enemies, they played a significant role in suppression of aphid in wheat crop.

VECTOR ROLE OF COCKROACH (*PERIPLANETA AMERICANA*) IN PROTOZOA, HELMINTHES AND PATHOGENS OF ENTERIC ORIGIN

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Cockroach are very much commonly present in our premises and frequently interact with humans. They can be found in sewerage, washroom, grain store and even can access our kitchen in search of food. They have adopted themselves as omnivores. Their ubiquitous presence made them to disseminate various pathogens including protozoa like giardia, entamoeba and helminthes including *Enterobious vermicularis*, *Ascaris lumbricoides* ova and bacteria of enteric origin like Salmonella, Klebsella, Strptococcus spp. In this study we have found the transmission role/ vector role of commonly found cockroach (*Periplaneta americana*). Fifty cockroaches (25 male and 25 females) were included in the study and their various body parts were separately dipped/ crushed for parasitological investigation and bacteriological exploration and for this purpose they were dipped in nutrient broth and incubated at 25°C for 24 hours. The growth obtained was further incubated and tested for various biochemical tests for the identification of pathogens on DESTO® strip test. The parasitological investigation results showed the presence of cysts of Giardia and Antamoeba along with eggs of *Ascaris lumbricoides* (unfertilized) and presence of Shigella bacteria. Further investigation on molecular level is advised.

GRASSHOPPER FAUNA OF DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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This study was conducted to know about the grasshopper fauna of district Swabi. Sampling was done from four tehsils of district Swabi. ie Swabi, Topi, Lahor and Razar. Each tehsil had three collection points. From tehsil Swabi collection was done from Shamansor, Anbarand Kunda and from the famous graveyard of Swabi. From Topi: Jadoon, Bamkhel and Ghazi were the collection site and from lahore specimens were collected from Manki, Hand and Haryan. From Razar the collection site was Dagi, Yarahussainand Ghohatee. The sampling was done regularly two to three times in a month. The sampling timing was as at morning and sampling was done on first and fifteen date of each month. I collected 334 specimens from the district Swabi from their four main tehsils. Specimens were identified by using key described in Shahid (1964) and we found 28 species, 18 genera, 10 subfamilies and 3 families namely as acrididae, pygomorphidae and tetrigitidae.

STUDY ON THE PYGMY GRASSHOPPERS (TETRIGIDAE: ORTHOPTERA) FROM SUKKUR DIVISION

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These insects are the smallest representatives of the Orthoptera, smaller less than 20 mm, mainly characterized by having highly elongated, tapered pronotum, that extends over the length of abdomen. Tagmina when present small and scale like and may be exposed or covered by pronotum. Members of the family Tetrigidae may vary in colors, brown grey, rusty grey or moss green in color and can be related to true grasshoppers. Pygmy grasshoppers usually found near water bodies, such as ponds, and streams. Occasionally they also found in dry habitats, woodlands, rice fields and in sandy areas with lichen. These grasshoppers especially eat roots of plants, seedlings, mosses, fungi, algae and cause considerable damage to crops. During the present study 03 species have been reported, pertaining to 02 genera i.e, *Euparatettix* Hancock, 1904, *Ergatettix* Kirby 1914 and species belong to these genera are *Euparatettix indicus*, (Bolivar, 1887) *E. sagittata* (Bolivar, 1887) and *Ergatettix dorsifera* (Walker, 1871). The specimens were collected from grasses, rice fields, plant roots, seedlings and from the boundary of a water pool and the stones with thick mosses growing on them, at the various places of Sukkur division. While two species of genus *Euparatettix*, *E. indicus*, (Bolivar, 1887) and *E.sagittata* (Bolivar, 1887) have been reported for the first time in the region of Sukkur division.

NEW RECORD AND DESCRIPTION OF TWO SPECIES OF GENUS *SPHAEROPHORIA* ST. FARG. ET SERV (DIPTERA: SYRPHIDAE) FROM BALOCHISTAN, PAKISTAN

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Hoverflies commonly known as flower flies belong to large family of small to large flies worldwide about six thousand species have been described. The present study was carried out from March 2016 to April 2017 in order to know that which species of hoverflies are found in this region. The specimens of genus *Sphaerophoria* St. Farg. et Serv were collected from different localities of Balochistan, using insect hand net with fine mesh. Two species namely *Sphaerophoria* i.e. *rueppellii* Wiedemann, 1830 and *Sphaerophoria interrupta* Fabricius, 1805 were recorded. These species are described here on the bases of their morphological characters especially their coloration coloration as well as on the basis male and female genitalia. This is the first time record of these species and is a new addition in literature of the insect fauna of Balochistan, Pakistan.

COLLECTION AND IDENTIFICATION OF BUTTERFLY FAUNA OF DISTRICT SWABI

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The Butterflies (Lepidoptera) have great economic importance in the World. The present survey was arranged to explore the butterfly fauna of District Swabi, Khyber Pakhtunkhwa. Butterflies were collected from various localities (Tarbela Dam, Topi, Maini, Pabaini, Marghuz, Maneri) of District Swabi every week using sweep net from June to August 2015. A total of 120 specimens were collected from mentioned locations respectively. The collected specimens were then identified with the help of available keys. The identification revealed that there are 11 species of Butterflies (Lepidoptera) under 3 sub families (Nymphalidae, Pieridae, Papilionidae). The species were *colias croceus*, *Danus chrysippus*, *Pieris napi*, *Junonia orithya*, *Pontia daplidice*, *Cynthia cardui*, *Pieris ajaka*, *Eurema hecabe*, *Papilio demolus*, *Neptis hylas*, *Catopsila florella*.

ECOLOGY AND DIVERSITY OF GROUND BEETLES (COLOEPTERA: CARABIDAE) IN PLAIN AND ROCKY AREAS OF SINDH PAKISTAN

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Carabid are generalist predator with shiny black metallic colour, composed of about 40,000 species distributed worldwide except Antarctica, Tasmania and smaller oceanic islands and atolls. Ecology and diversity of Ground beetles (Coloeptera: Carabidae) fauna were studied in two different regions plains and rocky of Sindh Pakistan during January to December 2018. Specimens were captured by hand picking and light trap method while a total of 1233 specimens belonging to 6 species and 5 genera of carabidae family were collected among which, *Nesamblyops oreobius*, *carabus nemoralis*, *Calosoma sycophanta*, *calosoma auropunctatum* were prevailing species where as *Harpalus suensoni*, *Mecyclothorax cordicollis* were least in number during study period. The abundance of carabid fauna varied seasonally, the highest number was recorded in spring and summer while lowest in winter. Species abundance correlation with temperature, humidity and rainfall were statically analysed. Habitat wise variation in average population size, species richness, evenness and diversity index were also calculated. The highest and lowest degrees of population size, richness and evenness and diversity were recorded at marshy and moist places.

IDENTIFICATION OF SEED BUGS (HEMIPTERA: LYGAEIDAE) OF TANDOJAM

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For present studies, specimens of Lygaeidae were collected from various localities of Tandojam. Further examination and identification was carried out at Insect Systematic Laboratory, Department of Entomology, Sindh Agriculture University Tandojam. Collection was made through sweep net, pooter and on light trap from various localities of Tandojam. To identify the specimen up to the species level, keys for the region were collected from various publications. Checklist was prepared from previous literature and further was updated with collection. In present study total 196 specimens of Family Lygaeidae Schilling, 1829 were collected from Tandojam. During the course of identification the material revealed the occurrence of 09 species under 3 subfamilies. Subfamily Lygaeinae Schilling, 1829 was discovered with the record of 7 species including; *Spilostethus hospes* (Fabricius, 1794), *Spilostethus pandurus militaris* (Fabricius, 1775), *Spilostethus simla* (Distant, 1909), *Graptostethus servus* (Fabricius, 1787), *Oxycarenus hyalinipennis* (Costa, 1843), *Karachicoris* sp. and *Cosmopleurus fulvipes* (Dallas, 1852). Subfamily Orsillinae Stål, 1872 with one species record; *Nysius* sp. and lastly subfamily Geocorinae Dahblom, 1851 was discovered with *Geocoris ochropterus* (Fieber, 1844).

SUSCEPTIBILITY OF DOMESTICATED ANIMALS FOR TICK (ACARI: ARGASIDAE) POPULATION IN TEHSIL SHUJABAD, PAKISTAN

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The prevalence of tick is a major threat to livestock that causes severe economic losses in animals. It spread many contagious diseases in animals and human like Crimean Congo Hemorrhagic Fever (CCHF) and Lyme Disease. The current study was carried out to check the tick infestation ratio on livestock in Tehsil Shujabad, during March - November 2018. Ticks were collected from selected parts like tail, udder and ears of cattle, buffaloes, goats and sheep, respectively. During study, it has been observed that tick infestation rate was highest in cattle (44.92%)

followed by buffaloes (26.08%), sheep (15.94%) and goats (13.04%). Further, the study showed that 41.60%, 33.87% and 23.87% ticks were recorded from cattle's tail, udder and ears, while on buffaloes, infestation rate was 50%, 27.77% and 22.22%, respectively. The percentage of tick infestation was high in sheep as compared to goats. It has been shown that tick infestation was highest in hybrid breeds in cattle.

MOSQUITO SPECIES DIVERSITY IN TREE HOLES IN MULTAN

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Mosquitoes are one of the important arthropod among the medically known insects. These creatures are responsible to spread different diseases in the human community like Malaria, Yellow fever, Zika and Dengue fever. In the present scenario, climate change and human activities are acting as a tool in its spreading. Thus to identify possible species and habitats survey was conducted in Multan area. During the survey, mosquitoes were found to be harboring in various tree species. The mosquito larvae were collected for thrice in a month. The collected insects were stored in 70% ethyl alcohol for subsequent identification. Results revealed that mosquitoes were found in correlation with Mangoes, Keekar and Sheesham. The dominating genera in the tree holes belong to the genus *Aedes* and *Culex*. Thus, the study will help the in better management of the vectors and in understanding their role in disease spread.

BIODIVERSITY OF ACRIDIDAE: ORTHOPTERA OF HAZARA DIVISION KPK PAKISTAN

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Cotton is one of the most important crop in the world. Its lint is used in textile and seed is crushed for oil and for planting next crop. It is self-pollinated crop but opportunistic. About ten percent out crossing occurs in cotton. A study was carried out at MNS-University farm in 2017. Two varieties of cotton including FH-Lalazar and FH-142 were grown in two blocks. Each block was divided into irrigated and drought. Two treatments including open pollination and self pollination were compared. Results showed that the abundance of pollinators were high in irrigated blocks of each variety. The results were significant in case of Seed cotton weight /boll, no. of seed/boll, seed weight, lint weight and GOT% in irrigated open plots while non-significant in drought self plots. Proper irrigation for the pollination of cotton was suggested.

A REVIEW OF THE GENUS *CHLOEBORA* SAUSSURE (1884) (OEDIPODINAE: ACRIDIDAE: ORTHOPTERA) FROM PAKISTAN

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The genus *Chloebora* Saussure is reviewed and a key to the species are given. Diagnosis of all the previously known species from Pakistan is given. The present investigations has been carried out on the material collected from the Sindh, Balochistan, Punjab and Khyber Pakhtunkhwa provinces of Pakistan. The following species were identified namely, *Chloebora crassa* Walker (1870), *Ch. grossa* Saussure 1884 and *Ch. bramina* Saussure 1884. This last species is recorded for the first time from Pakistan.

SURVEY OF THE EXTERNAL PARASITE TICKS (ARTHROPODA: ARACHNIDA: ACARI: IXODIDA) ON COMMON QUAIL (*COTURNIX COTURNIX*) OF PAKISTAN

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Parasites are important subjects to study in the animal and veterinary health sciences. Parasitic diseases are considered as a major obstacle in the well being and product performance of animals. It can be due to the endo-parasites that nourish inside the animal bodies, or the ecto-parasites or external parasites such as ticks, lice, mites, flies, fleas, midges, etc., which attack the external body surface to get food. Among ecto-parasites, ticks are very important and harmful blood sucking external parasites of mammals, birds and reptiles throughout the world (Furman and Loomis, 1984). The medical and economic importance of ticks had long been recognized due to their ability to transmit diseases to humans and animals. Ticks belong to phylum, Arthropoda and make up the largest collection of creatures in order Acarina. Ticks are divided into two groups: soft bodied ticks (Argasidae) and hard bodied species (Ixodidae). Hard ticks feed for extended periods of time on their hosts, varying from several days to weeks, depending on such factors as life stage, host type, and species of tick. The outside surface, or cuticle, of hard ticks actually grows to accommodate the large volume of blood ingested, which, in adult ticks. (Sonenshine, 1991). Ticks are also found on the common quails (*Coturnix coturnix*). Ticks from the genus *Amblyomma* and *Haemaphysalis* have already been published occurring on bobwhites and scaled quails but no one has studied the prevalence of ticks on common quails (*Coturnix coturnix*). From this study, 20 quails were shuffled to trace and obtain the ticks and yet two species have been recovered which are under the process of identification as the study has just started to be continued. The conclusion is that ticks cause big economic loss to *coturnix coturnix* globally. Ticks also transmit a wider variety of pathogenic agents while as the vaccine is not available easily in Pakistan. Therefore, there is more need to work on this topic.

POPULATION DYNAMICS OF COTTON JASSID (*AMRASSICA BIGUTTULA*) IN RELATION WITH ABIOTIC FACTORS IN MULTAN

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Cotton (*Gossypium hirsutum* L.) is one of the important crops globally and known as the white gold. In Pakistan, it is one of the major cash crops. However, the production of cotton is not being increased within the several years. It is mainly because of being attacked by the sucking insect fauna especially by the cotton Jassid. Thus, the present study was carried out to know about the seasonal fluctuation of Jassid. Data was collected from July – October in Multan, 2017. During the study, Jassid population was noted on two genotypes these are *Bt* (MNH-992) and non-*Bt* (Cyto-124). The impact of weather parameters like temperature, humidity and rainfall on cotton jassid was recorded. The results revealed that the minimum population of Jassid was observed on 2nd week of July and the pest population was 0.83 and 0.92 per leaf, while the maximum population was recorded during the 1st week of September *i.e.* *Bt* (10.25 individuals per leaf) and non-*Bt* (9.35 individuals per leaf) respectively. The correlation of Jassid population showed significant effect with the temperature and rainfall, while the relative humidity has non-significant effect. Thus, it can be concluded that the climate change has prominent effect on the Jassid population.

**PREVALENCE AND MORPHOLOGICAL IDENTIFICATION OF RHIPICEPHALUS TICKS
COLLECTED FROM DIFFERENT SITE OF CATTLE POPULATION IN DISTRICT
KARAK KHYBER PAKHTUNKHWA**

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Ticks and tick-borne infections pose major economic threats to the livestock industry. Particularly in Pakistan. The present study was conducted at the three Tehsil i.e. Tehsil Karak, Tehsil Banda Daud Shah and Tehsil Takhti Nasrati of District Karak to determine the prevalence and identified morphologically the Rhipicephalus ticks among the collected ticks from cattle population. A total of 1240 ticks were collected from 400 cattle population including Cows, Bull and Calf, were examined on a stereo microscope, prevalence and morphological identification were made by using standard keys. The prevalence rate of the Hard ticks were found 69.25% (277/400), among these Rhipicephalus microplus 29.25% (117/400) followed by R.appendiculitidis 15.25% (61/400), R.decoloratus 7.75% (31/400) and mixed infestation of ticks 17% (68/400) in the cattle population. The sex wise prevalence was recorded and found that male 52% (39/75) and female (cow) were recorded 73.23% (238/325) in the research areas of district Karak. The age wise prevalence was recorded during the study and found that adult 66.45% (166/250) and young were found 74% (111/150) of cattle population were infested with ticks in three Tehsil of district Karak Khyber Pakhtunkhwa. The morphological identification among 400 cattle population including Cows, Bull and Calf were 69.14% (857/1240), among these were Rhipicephalus microplus 29.19% (362/1240), followed by R.appendiculitidis 15.25% (189/1240), R.decoloratus 7.74% (96/1240) and Mixed infestation 16.93% (210/1240). It was concluded from the study that R.microplus ticks were more prevalent in cattle population and become a health hazard to livestock as well as to the human population. The female cows were more infested then male in the study areas.

**MODERN TAXONOMIC TOOL BY USING Cyt-B AND Pol GENE FOR
THE EVOLUTIONARY STUDY OF WASP**

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Present study describes the phylogenetic reconstruction of wasp species found in the Hazara region of Pakistan. The investigation was based on nucleotide sequence analysis of specific markers: Cyt-B and Pol genes. A total of 2500 samples were carried out which represented 19 species from 3 subfamilies including: Eumeninae: six species, Polistinae: six species and subfamily Vespinae: seven species. Using partial sequences of Cyt-B gene, *D. conoideum*, *D. esuriens* showing the same group 100% of homology, whereas *A. a. bangalensis*, *D. dimidiatipenne* 86% and *A. flavescens*, *R. quinquecinctum* 79%. In Polistinae species, *P. indicus*, *P. wattii* showing 100%, *P. olivaceus*, *P. rothneyi* 90-89%, whereas *P. stigma* 87%. *Vespa mandarina*, *V. orientalis* representing the same group 100% homology, whereas *V. analis*, *V. basalis* 84% and *Vespa tropica* 81% subfamily Vespinae. With the use of partial sequence of Pol gene, species of the subfamily Eumeninae, *A. a. bangalensis*, *R. quinquecinctum* showing 96% homology, whereas *D. conoideum* 35% representing an out group. Members of the subfamily Polistinae, *P. indicus*, *P. wattii*, *P. olivaceus* showing 100% homology, whereas *P. rothneyi*, *P. stigma* 99% and *Ropalidia brevita* 95%, subfamily Vespinae, *V. analis*, *V. orientalis* 100%, whereas *V. mandarina*, *V. tropica* 99%. The result shows nine species: *Delta conoideum*, *D. esuriens*, *Anhynchium abdominale bangalense*, *Rhynchum quinquecinctum*, *Antodynours flavescens*, *Ropalidia brevita*, *P. olivaceus*, *P. stigma* and *V. mandarina* were new record for Hazara region whereas four species: *V. mandarina*, *Anhynchium abdominale bangalense*, *Rhynchum quinquecinctum* and *Antodynours flavescens* were new record from Pakistan.

EXPLORING THE DRAGONFLY FAUNA OF SWAT VALLEY, PAKISTAN

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This study was based on the exploring Dragonflies fauna of District Swat. For this purpose a total number of 2140 samples were collected from the different localities: of Swat valley. The collected samples were preserved by using insecticides spray (Black cobra), and transfer into zoological lab (CAS&F) center for animal sciences and fisheries. Morphological study and identification was carried out by using taxonomical keys of the British Dragonflies society journal (BDS). The study yielding two families *libellulidae* and *Aeshnidae* and 8 genera with 13 species (*Aethriamanta aethra*, *Orthetrum ransonjneti*, *Palpopleura sexmaculata*, *Crocothemis erythraea*, *Orthetrum chrysis*, *Crocothemis sanguinolenta*, *Acisoma panorpoides*, *Ladona deplanata*, *Crocothemis servilia*, *Aethriamanta brevipennis*, *Orthetrum abbotti*, *Indothemis limbata*). During this study one species were noted from family *Aeshnidae* and remaining 12 species were belonging to family *Libellulidae* the results show that the most abundant family is observed *Libellulidae* and the most abundant Species were reported *Aethriamanta aethra*.

MANAGEMENT OF BOT FLY (DIPTERA: OESTRIDAE): A SERIOUS THREAT TO LIVESTOCK SECTOR WORLD WIDE

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Livestock is an important industry having great economic value worldwide since it is a major source of nutrients (eggs, milk and meat) and good source of earning for low income people. Various diseases and pests cause severe losses to livestock industry and among these bot fly is an emerging threat across the world because of unawareness and negligence of the stakeholders involved. This fly is also known as warble fly that has been reported to infest 100% camel flocks in Ethiopia. Bot flies are the true flies belong to order Diptera and family Oestridae having 160 species worldwide. Adult female of bot fly lay eggs on the host or its surrounding in order to easily enter inside the host body through its naturally openings followed by feeding on the tissues or body mass of animals and cause myiasis disease. It has been reported to consume the 5% body mass of the host (as per host weight). Several attempts have been made to rear it under in-vitro conditions but none is succeeded. Infested animals show various symptoms of its attack i.e. aggressive behavior, sneezing, snorting, and neurological, respiratory and digestion disorder. Moreover, its infestation causes reduction in milk production, growth rate and weight along with damaging the hides of the animal while in severe cases mortality of the host occurs. Bot fly infestation has severe economic impacts as an estimate from Brazil has reported US\$200–260 million losses annually. Among the various management techniques of bot fly, various chemicals (Ivermectin, Doramectin, Moxidectin, Abamectin) have been found effective against bot fly infestation at recommended doses. However, non-chemical control methods i.e. biological control (*Beauveria tenella*), along with mechanical and cultural practices have also shown promising results for its management.

THE PREVALENCE OF *HYALOMMA* TICK INFESTATION IN LIVESTOCK OF DISTRICT LAKKI MARWAT, KPK, PAKISTAN

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Ticks are the main hematophagous arthropods and obligatory ectoparasites that are considered a vector of serious pathogens for animals and humans. Ticks and tick borne infections impose major economic threats to the livestock industry throughout the world. The present study was carried out from December 2017 to April 2018 with the objective of determining the prevalence of *Hyalomma* tick infestation in livestock of District Lakki Marwat, KPK Pakistan. The sample was collected by handling from different livestock in District Lakki Marwat and preserve in alcohol. Ticks were identified on the basis of morphological features. The overall prevalence of *Hyalomma* tick infestation during the study period was found to be highest in buffaloes (69.69%), followed by cattle (49.76%), sheep (36.40%) and goat (18.01%). The prevalence of *Hyalomma* ticks was found to higher (46.05%) in the age of above than 5 years followed in order by 1-5 years age (42.11%) and 1 year (27.8%) livestock. Sex wise prevalence of *Hyalomma* tick infestation highest in females (42.62%) than males (31.39%). In different areas of Lakki Marwat different infestation rate was found in which Ahsanpur show the highest prevalence 63.97% as compared to Ghazni Khel 28.94% and Titter Khel 27.05%. It is suggested that highly modified strategy are required to control the *Hyalomma* tick infestation in livestock to increase the livestock yields and reduce the economic losses.

DISTRIBUTION OF MOTHS FAUNA IN JAMSHORO SINDH, PAKISTAN

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Moths are Polyphagous in nature larvae and adult are phytophagous. Larvae of many species are serious pest of agricultural crops. Present Study was carried out in Jamshoro district from March to December 2018 Adult Moths collected through light trap hand picking and insect net. Moths activity started during the 4th week of March reaches to its first peak during the 2nd week of April and drastically decline up to the end of May. No Moth was captured during June and July. Total 4 species *Acherontia Styx*, *Cnaphalocrocis medinalis*, *Utetheisa pulchella*, and *Chalciope mygdon*, belonging to different families were collected.

DISTRIBUTION OF GEOMETER MOTHS (GEOMETRIDAE: LEPIDOPTERA) FROM DIFFERENT AGRICULTURAL AREAS OF JAMSHORO

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Moths are nocturnal in nature and comprising 95% of lepidopteran insects. They are distributed throughout in world mostly tropical and sub tropical parts. In Pakistan many species of moths are reported out of these some reported serious pests of agriculture crops such as cotton, rice, sugarcane and vegetables. Present study was carried out from March 2018 to December 2018 in different agricultural areas of jamshoro, Adult moths were collected by different methods light traps, hand picking and insect net. During present study 3 species of geometer moths of family Geomatridae belonging to one genera were collected. Adult Moth species collected from different host plants. Maximum population was recorded during March while lowest population recorded in the month of June and December.

OCCURRENCE BUTTERFLIES AND MOTH IN VEGETABLE FIELDS OF DISTRICT THATTA

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Lepidoptera is one of the largest order of insects that includes moths and butterflies. It is one of the most widespread and widely recognizable insects in the world. The term was coined by Linnaeus in 1735 and is derived from Ancient Greek (scale) and (wing), comprising an estimated 174,250 species, 126 families and 46 super families. Presently different vegetables fields of Thatta district were survey for the collection of butterflies and moths. Total 143 specimen were collected from different vegetables, out of these 129 were butterflies and 14 were moths. After collection these specimen were brought to Entomology laboratory Department of Zoology for preservation and identification process. These specimen were preserved and kept them in to insect box. Butterflies and moths were collected from Chickpea, Cabbage and milkweed plant. Total 3 species of Butterflies *Danaus chrysippus*, *Junonia almana*, *Colotis amata* and 1 specie of moth *Helicoverpa armigera* were collected.

SAND FLIES; EMERGING INSECT DIVERSITY IN THE MULTAN

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The recognition of medically important arthropods is an important step in determining the possible disease outbreak in the human communities. This is due to the high rate of urbanization which forced the medical insects to live in the modified environments of humans thus causing problem. So, keeping the scenario, diversity of sand flies was accessed. Data was collected solely from the Multan area once in a month. Sand flies were captured with the help of trap made by castor oil coated on white paper. The collected sand flies were stored in 70 % Ethanol in Ecology Lab of MNS-University of Agriculture, Multan. Prior to identification, flies were treated with KOH and then identified by using the morphological key. The results revealed the presence of the two major genera i.e. *Phlebotomus* and *Sergentomyia*. The identified species are *Phlebotomus papatasi*, *Sergentomyia christophersi*, *Phlebotomus colabaensis*. The study will help in better understanding the role of vectors in disease transmission and identification of species.

TAXONOMY AND DISTRIBUTION OF SAND FLIES IN MIRPUR DIVISION AJ&K

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Phlebotomine sand flies are small sized (1.5-2.0mm) hematophagous insects that have the ability to serve as vectors of various diseases, including leishmaniasis. The present study was designed for the taxonomy and distribution of sand flies in Mirpur Division, Azad Jammu & Kashmir. A total of 293 sand flies were captured from 28 localities using sticky paper traps from July, 2017 to December, 2018. *Phlebotomus* and *Sergentomyia* were two genera of sand flies identified from captured specimens. Five species of genus *Sergentomyia* were identified viz. *Sergentomyia* (*Parrotomyia*) *babu babu annandale*, *Sergentomyia* (*Parrotomyia*) *baghdadis*, *Sergentomyia* (*Sintonius*) *christophersi* (*Sinton*), *Sergentomyia* (*Sergentomyia*) *punjabensis* and *Sergentomyia* *baili* (*Sinton*). *Phlebotomus* (*Paraphlebotomus*) *alexandri* *Sinton* and *Phlebotomus* (*Euphlebotomus*) *argentipes* were two species of *Phlebotomus* genus identified from captured specimens. The abundance of *Sergentomyia* genus (93.52%) was higher in comparison to *Phlebotomus* genus (6.48%). The average number of captured sand fly specimens was higher in postmonsoon season (mean=3.307±5.01) as

compared to monsoon season (mean=0.133±0.345). Majority of sand flies (n=240) were captured from vegetation in comparison to other selected habitats (such as bird's cages, animal's herds and indoor places). The present study indicates the existence of diverse sand fly fauna in Mirpur Division AJ&K.

EFFECT OF BIOLOGICAL FAUNA ON THE WHEAT APHID

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Wheat is an important and widely grown crop in Pakistan. Also the same is known to be one of the major cash crops in the country. However, the wheat is being attacked by the aphids which results in its low production. In this scenario, the efficiency of biological agent was accessed. The study was conducted on the wheat crop from November – March, under the research trial of MNS University of Agriculture, Multan. During the study, biological agents were used to access the population density of aphids. The beneficial insect that was used in the study are Spiders, Staphylinid beetles, Coccinellid beetles and Syrphid flies. The data was recorded thrice in a week. The results revealed that Coccinellid beetles are dominating among the other beneficial insects. Also, the collective preying by the biological fauna resulted in the decrease of 75 % aphid population in the wheat crop. Thus, it can be concluded that aphid can be controlled by using the biological fauna.

A NEW SPECIES OF POTTER WASP OF GENUS *SUBANCISTROCERUS* (HYMENOPTERA: VESPIDAE: EUMENINAE) FROM PAKISTAN

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Potter wasps belong the subfamily Eumeninae. These wasps are mud daubers i.e construct their nests with mud or clay, solitary in nature and help in biological control of insect pests. Potter wasps were collected from four districts of Multan, namely Vehari, Multan, Lodhran and Khnewal. Two hundred specimens of potter wasps were collected with the help of ariel nets and spot catching. Out of 200 collected specimens a new species of potter wasp, *Subancistrocerus pakistanensis* Qasim, Carpenter et Rafique, sp. nov. identified including three Paratype. This species is belonged to genus *Subancistrocerus*, which is also recorded first time from Pakistan. Species of this genus belong to Oriental Region.

VESPIDAE (HYMENOPTERA) CATALOG OF MULTAN REGION, PAKISTAN

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Wasps are diverse and important group of insects, which decline important insect pests of agriculture crops. They play vital role in the ecosystem. During study wasps were collected with the help of ariel nets from various

localities of Multan Region. From collected specimens a total of 14 species from 10 genera under subfamilies Eumeninae, Polistinae and Vespinae are listed. The subfamily Eumeninae is represented by 11 species in eight genera, while two species of genus *Polistes* and one species of the genus *Vespa* represented subfamily Polistinae and Vespinae respectively. Among these *Allorhynchium argentatum* (Fabricius 1804), *Antepipona ceylonica* (de Saussure 1867), *Delta esuriens esuriens* (Fabricius 1787), *Delta pyriforme pyriforme* (Fabricius 1775), *Odynerus reniformis* (Gmelin 1790), *Xenorhynchium nitidulum* (Fabricius 1798) of subfamily Eumeninae, *Polistes indicus* Stofa 1934, *Polistes wattii* Cameron 1900 under subfamily Polistinae and *Vespa orientalis* Linnaeus 1771 under subfamily Vespidae are recorded first time from Multan Region. Genus *Odynerus* Latreille 1802 and two species: *Antepipona ceylonica* (de Saussure 1867) and *Odynerus reniformis* (Gmelin 1790) of subfamily Eumeninae are new records for Pakistan.

EFFECT OF DROUGHT STRESS ON POLLINATOR VISITATION AND SEED YIELD OF CANOLA (*BRASSICA NAPUS*)

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Climate change is seriously affecting the phenology, distribution and physiology of various groups of invertebrates. Therefore, its impacts on plant pollinators interactions should also be evaluated keeping in view the vital role of pollinators in maintaining plant diversity and ensuring global food security through crop pollination services. Drought stress is one of the primary and main part of climate warming increasing across various regions worldwide. In order to determine the effect of drought stress on the abundance and visitation rate of insect pollinators along with seed yield of canola (*Brassica napus* L.), an experiment was carried out at the research farm of Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan. Twelve insect species belonging to two orders and five families were found visiting canola flowers in drought and irrigated plots. It was found that abundance and diversity of insect pollinators was significantly higher in irrigated plots than the drought plots. The foraging behavior (stay time and visitation rate) of insect pollinators also varied significantly in irrigated and drought plots. Moreover, there was also significant difference in yield attributing factors (pod weight, seed weight per pod, no. of seeds per pod, number of pods per plant) in open pollinated irrigated plots than the drought plots as a result of higher pollinator abundance and visitation rate in the former ones. Keeping in view the current climate change scenarios and limited water availability, future studies should consider this aspect in other cross pollinated crops under varying environment and insect fauna.

POPULATION DYNAMICS OF CEREAL APHIDS IN WHEAT CROP AT DISTRICT SWABI

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Being serious threats to cereal crops, the estimation of aphid population and its timely control is always a challenge for agriculture scientists. In order to evaluate population dynamics of aphid a survey was conducted in selected areas of district swabi. For population dynamics, number of aphids were recorded on weekly basis using diagonal methods by randomly selected 5 plants from each field. The findings of the study indicated that aphid attack started in the 1st week of February and increased as the vegetative growth proceeded. Moreover, the aphid population was higher in the 2nd and 3rd week of March and decreased subsequently.

ASSESSMENT OF VARIETAL PREFERENCE OF APHID ON WHEAT GENOTYPE UNDER AGRO-ECOLOGICAL CONDITIONS OF SARGODHA, PAKISTAN

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Wheat (*Triticum aestivum* L), is the most important staple food crop under cultivation in Pakistan. It plays major involvement in the agricultural economy of country. The yield of wheat is reduced than other countries, due to many factors like lack of irrigation facilities and poor varieties. Several insect pests attach wheat but aphid cause severe damage. Aphid directly (35-40%) and indirectly (20-80%) causes damage by sucking the cell sap and transmit viral and fungal diseases. The present study was conducted from Dec 2017-April 2018 to evaluate the seasonal population fluctuation of aphid on two wheat varieties like Lasani-08 and Faisalabad-08 at the College of agriculture, university of Sargodha, Pakistan. The maximum population (35 aphids/ tiller), and (15.20 aphids/tiller) was observed on Lasani-08 and Faisalabad 08, respectively during the 2nd week of march 2018, while the minimum population (3.78 aphids/tiller) and (1 aphid/tiller) was on Lasani-08 and Faisalabad-08 during 3rd week of April-2018. The result showed that the lasani-08 is more susceptible to aphid's infestation than Faisalabad-08. The impact of weather parameters like rain fall were also recorded. The rainfall had negative and significant correlation with the aphid populations.

BIODIVERSITY AND DISTRIBUTION OF BUTTERFLIES IN DISTRICT BAGH

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Present study to explored the biodiversity of mosquitoes (Diptera: Culicidae) were carried out in district Bagh and some adjoining areas for the study period of six month (May-October). This is the first attempt to find out the biodiversity of Mosquitoes from district Bagh. The Mosquito fauna was explored from undisturbed buildings, tree hole and stagnant water bodies. Mosquito fauna was collected by using mouth aspirator and hand net. After collection specimens were pinned up and stored in entomological box. Then the collected specimens were identified under the microscope by following the taxonomic keys of Barraud (1934); Rueda (2004) and Becker(2010).The study work will provide the base line date for further researcher.A total of 2895 specimens of mosquitoes were collected from the study area belonging to order Diptera, family Culicidae and two subfamilies, anophilinea and culicinae. During this research total eleven species were identified one species; *Anopheles bariensis* which is belong to family anophilinae and genus Anopheles.Seven species; *Culex pipiens*, *C. epidesmis*, *C. pseudovishnui*, *C. fuscocephala*, *C. fatigan*, *C. vishnui* and *C. barraudi* belong to subfamily culicinae and genus culex.Two species such as *Aedes aegypti* and *A.micropterus* belong to subfamily culicinae and genus aedes. One species *Armigeres subalbatus* belongs to subfamily culicinae and genus Armegerus .The most abundant species was *Armigeres subalbatus*.

BIODIVERSITY AND DISTRIBUTION OF BUTTERFLIES IN DISTRICT BAGH**Rafia Sajjad¹, Muhammad Shahbaz^{1*}, Seyda Najaf¹ and Arshad Javid²**¹ Department of Zoology, Women University, Azad Kashmir, Bagh, Pakistan² Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan

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Butterflies are distributed all over the world, are easy to identified and sample them, as species as well as individuals. Their significant number is present in different areas (Sawchik *et al.*, 2005). During this short term study extending from March to October, 2017. Efforts were made to explore the butterfly fauna of district Bagh. Specimens were captured with the help of hand net and insect net. Captured specimens were identified by comparing with available literature and following the keys of Abbas *et al.*, 2002) and (Muniret *et al.*, 2007). During this study, more than 200 specimens belong to three families, nine genera and (13) species of order Lepidoptera, Family Pieridae: Genus: *Eurema* *E Nhecabe*, Genus: *Colias*; *C. erate*, Genus: *Pieris*: *P rapae*, and *P. canidia*, Genus: *Leptidea*; *L. sinapis*; Family: Nymphalidae: Genus: *Junonia*: *J. orithy*, Genus: *Phalanta*: *P. phalantha*, Genus: *Aphantopus*: *A. hyperantus*, Genus: *Limenitis*: *L. weidemeyerii* and Family: Papilionidae: Genus: *Papilion*: *P. polytes*, *P machaon*, *P demoleus* and *P.Ulysses*. This study will provide baseline data to future researchers. identified specimens were labeled and entry was made in the diary, place of collection and the time of collection were also noted. This study will be helpful for the future policy makers, planners and researchers working on biodiversity of butterflies in District Bagh.

BIODIVERSITY AND DISTRIBUTION OF CALLIPHORIDAE (BLOW FLIES) IN DISTRICT BAGH, AZAD KASHMIR, PAKISTAN**Muhammad Shahbaz^{1*}, Seyda Najaf¹ Arshad Javid², Abu ul Hassan Faiz¹**¹ Department of Zoology, Women University, Azad Kashmir, Bagh, Pakistan² Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore

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Blow flies are generally known as green bottles and blue bottles belong to family *Calliphoridae*, is a big family, with five subfamilies, nine genera and sixteen species. To explore the biodiversity of blow flies, specimens were collected from different inhabiting sites, like from dead materials, heaps of garbage and fecal matter localities of the study area. This study was conducted from May to October 2017. For the collection of specimens of flies hand net and pyrethrum spray was used. After collection specimens was pinned up and stored in entomological box. A total of 240 specimens were collected from study area belongs to seven genera and 6 species. The collected specimen has been identified by following the available literature and taxonomic keys of White *et al.*, 1940. with the help of hand lens and dissecting microscope. The results revealed that a total of 7 species belonging to three genera and two sub families: Calliphorinae contains four genera: *Calliphora*; *C graham*, *Lucillia*; *L cuprina*, *L. ampullacea* and two genera *Hemipyrellia* and *Hypopygiopsis* remained unidentified. Sub family Chrysomyiinae: four species of genus *Chrysomyia* namely; *C. villeneuvei*, *C rufifacies*, *C nigripes* and *C albiceps*. This study was conducted to explored the fauna of blow flies and provide the base line data to future researchers for better epidemiological understandings.

STUDY OF PEACH FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE) LIFE CYCLE ON DIFFERENT HOST FRUIT PLANTS**Farwa Aslam*, Muhammad Ahsan Khan, Kiran Shehzadi* and Taskeen Ahmad**¹ Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad² Department of Entomology, University of Agriculture, Faisalabad

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Fruit flies (Diptera: Tephritidae) cause mostly damage to fruit plants. Eleven species of fruit flies have been discovered from Pakistan. *Bactrocera zonata* (Saunders) commonly called peach fruit fly is considered one of serious

polyphagous pest insect, which can attack more than 50 host fruit plants. Present experiment was conducted to study life cycle and biology of *B. zonata* on different host plants. Previous work on *B. zonata* is revolved around mass-culturing in laboratory for research purpose and parameters of growth and development are indicators of performance in various studies. Different population or eco-strains of *B. zonata* were cultured on different host fruit plants such as guava, mango, and peach separately to determine different development stages (larvae, pupae and adults). Quantitative parameters, viz., length, weight, were noted at each level of every stage. Parameters of survival, development, size of pupa and fecundity as well as population factors such as intrinsic rate of increase in population. Effect of temperature and humidity was also noted. Statistical analysis of the studies revealed that peach was the most suitable host for development and growth of *B. zonata*.

TAXONOMY OF FAMILY SALTICIDAE FROM SUGARCANE CROP DISTRICT NAWABSHAH, SINDH, PAKISTAN

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Pakistan is the fifth largest Sugar producer in the world (FAO,2009). District Nawabshah is most important agricultural district of Sindh, Pakistan. During the present study, Sugarcane field of district Nawabshah of four talukas namely Kazi Ahmed, Sakrand, Daur and Nawabshah were surveyed in the month of April 2017 to January 2018, 193 specimens were collected and from the all collected spiders 4 genera and 8 species and 1 new species were identified with the help of taxonomical identification keys, (Ursani TJ 2004) identified species during the study period are *Plexippus Paykulli*, *Plexippus Calcutaensis*, *Marpissa Sehwani*, *Marpissa Insignis*, and new species *Marpissa Benazirae*, and *Phiddipus Punjabensis*, *Menemerus Similibattus*, *Menemerus Bivittatus* these all and one new species were recorded from District Nawabshah.

HONEY BEE PROPOLIS: A POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS HAVING ANTIMICROBIAL PROPERTIES

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Bee propolis is a natural resinous product of bee hive. Honey bees collect plant resins, mix them with their own enzymes to produce propolis. It is used to fill small gaps of hives and for protection of bee hives from microorganisms and diseases. Chemical composition of propolis showed complexity of numerous organic and inorganic compounds including polyphenols, steroids, sesquiterpene quinines, phenolic aldehydes, coumarins and amino acids. Propolis showed a variety of biological and pharmacological properties such antimicrobial, antiviral, antiparasitic and antioxidant activities. Due to its antimicrobial property, propolis has gained much attention among of the researchers as a valuable natural product to fight against microbial infection. Propolis showed antimicrobial activities against gram positive and gram negative bacteria, viruses, parasites and other disease causing agents. Furthermore, it is used to enhance shelf life of food products as a natural preservative and its bioactive compounds have been recognized as safe for human consumption. In future, Propolis could be a good source of natural antimicrobial compounds those can be isolate and used in drug development and food preservation. Therefore clinical studies should be planned to investigate the role of propolis in disease prevention and health promotion to increase the commercial production and utilization of propolis.

PREVALENCE OF NASAL WORMS AMONG THE POPULATIONS OF GOATS IN SINDH, PAKISTAN AND ESTIMATION OF GENERAL PROTEIN THROUGH SDS-PAGE ELECTROPHORESIS

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Goat (*Capra hircus*) is a versatile animal and assumes an important function in the economy and sustenance of landless, little and minimal agriculturists in Pakistan. Goat infected with a spread of parasites. Among the various parasites, Nasal worm is of major concern. Nasal worms are the larval phase of an insect called *Oestrus ovis*, dipteran insect. These nasal worms can induce a myiasis throughout the world called nasal oestrosis. This infection frequently found in goats and sheep. The pathogenic effects lead to considerably less animal production and significant economic losses. Oestrosis additionally regarded as a zoonotic disease. The present Study was conducted in different areas of Karachi Sindh. Specimen collected from those Slaughterhouses and butcher shops, where the goat supply comes from all over the Sindh. A total of 527 (285 male and 242 female) goat heads was examined for the presence of *Oestrus ovis* larvae. Out of the examined, 191 were found infected, with a 36.24 % rate of infection. The infection rate in male goats was 39.64% that are higher as compared to female goats that is 32.23%. A total of 1434 larvae were collected from examined goat heads. The mean number of larvae in infected goats was 7.5. Density of *Oestrus Ovis* larvae in infected goat was changed from 1 to 40. The average weight (0.37g), length (1.69cm) and width (0.73cm) of larvae were calculated. During the current study, the protein-banding pattern in larvae of *Oestrus ovis* among the populations of goats in Sindh, Pakistan was estimated through SDS PAGE electrophoresis and the results revealed that the relative mobility of protein pattern showed differentiation.

ASSESSMENT OF DIVERSITY AND RELATIVE ABUNDANCE OF INSECT FAUNA ASSOCIATED WITH *TRITICUM AESTIVUM* BY USING QUADRATE METHOD FROM DISTRICT SIALKOT

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Agriculture performs an important role in the economy of Pakistan. Wheat is the leading grain food of Pakistan and provides stable diet for people. Insects can be important pests in agriculture, forestry and homes, and can threaten to human health. The insect population can reach high numbers and cause a significant amount of damage unless control measures are taken against them. During present study Wheat (*Triticum aestivum* L.) crop was sampled to assess the diversity and relative abundance of insect fauna by using quadrature method. Sampling period extended from February 2017 – June 2017. A total no. of 839 specimens was sampled in wheat crop of district Sialkot. Sampled specimens were found to belong from five orders, nine families and 15 species. Insects were belonging to order Coleoptera, Hemiptera, Hymenoptera, Lepidoptera and Diptera were collected. Month wise distribution of insect fauna was determined. Highest number of specimens were recorded in the month of March (490 specimens) followed by April (138 specimens), Feb (127 specimens), May (57 specimens) and June (27 specimens). *Shizaphis graminum* was the most dominant species contributing 57.3 % in the sampled data followed by *Spodoptera exigua* (54.01%) and *Spodoptera litura* (53.7%), *Coccinella septempunctata* (42.11%), *Musca domestica* (40.03%), *Culex pipiens* (17.38%), *Coccinella septempunctata*'s larvae (13.74%), *Coccinella septempunctata*'s pupae (12.15%), *Polistes olivaceus* (9.07%), *Apis cerana* (8.23%) and *Ischiodon scutellaris* (5.21%). Identified Insect fauna was assigned with guilds based on their feeding habits. Accordingly, three major insect guilds were identified that are carnivores, herbivores and omnivores. Predator-prey interaction of selected abundant species was determined by applying linear regression analysis. Significant interactions were found among preys and predators. The highest association was observed between *Cheilomenes sexmaculata* and *Shizaphis graminum* ($R^2 = 0.998$) followed by *Diuraphis noxia* ($R^2 = 0.994$) and *Rhopalosiphum padi* ($R^2 = 0.96$). *Coccinella septempunctata* showed association with maximum no. of selected prey's specimens. Significant differences were observed in diversity of insect fauna

among different months by applying Shannon Diversity Index. The impact of environmental factors on abundance and diversity of insect fauna was determined by canonical correspondence analysis. Such types of studies are necessary to design integrated pest management programs to control the pests.

**STUDY OF ECO-BIOLOGY, DIVERSITY AND RELATIVE ABUNDANCE OF INSECT FAUNA OF
ZEA MAYS (MAIZE) AND TRIFOLIUM ALEXANDRINUM (BERSEEM) BY USING SWEEP
METHOD FROM DISTRICT SIALKOT**

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Agriculture is a chief source of economy in Pakistan which is directly or indirectly associated with insect diversity as they act as important predators, pollinators or pests of agricultural crops. The present study aimed to assess the insect diversity of region Sialkot. The insects from different areas of district Sialkot were sampled by using Sweep method from *Zea mays* (Maize) and *Trifolium alexandrinum* (Berseem) crops. The insects were identified upto species level. A total of 946 and 805 specimens were sampled from maize and berseem, respectively belonging to six orders, 15 families and 29 species. Shannon Wiener diversity index was used to assess diversity of insect fauna from selected crops. Relative abundance of each of order family, genus and species was calculated, representing maximum abundance of order Orthoptera with 330 specimens in maize crop and order Coleoptera with 256 number of insects from berseem. Linear Regression analysis was applied to check the correlation among selected Predator and Prey species which can be used to predict possible prey and predators. Maximum correlation with R^2 value, ($R^2 = 0.81$) was observed between *Camponotus itoi* and *Rhopalosiphum maidis* in maize and *Coccinella septempunctata* and *Rhopalosiphum maidis* species showed maximum correlation ($R^2 = 0.95$) from berseem. Canonical Correspondence Analysis (CCA) was applied to check the effect of environmental factors on insect fauna, showing a positive correlation of species with these meteorological factors. Such type of studies is very helpful in designing Biological Control programs and Biological Control Programs are very valuable technique for (IPM) and further for sustainability of agro-ecosystem.

**EXPLORING THE ROLE OF APIS AND NON-APIS INSECT VISITORS TOWARDS CROSS
POLLINATION, THEIR INFLUENCE ON YIELD, OIL CONTENT AND OTHER QUALITY
PARAMETERS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) IN MARDAN, KHYBER
PAKHTUNKHWA**

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Field studies were carried out at Agricultural Research Farm, Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa during 2017. The trial was carried out to identify different insect visitors including *Apis* as well as non-*Apis* insect visitors associated with sunflower, to assess their visitation pattern and to determine their influence on yield increment, oil content and other quality parameters of the crop. A total of fifteen different visitors were associated with sunflower. Hymenoptera were the most abundant that made 87% of the whole population followed by Lepidoptera (8%) and Diptera (5%). The relative abundance of four major species, i.e. *Apis mellifera*, *A. dorsata*, *A. cerana* and *A. florea* were recorded on hourly and weekly basis. The peak densities of all honey bee species were recorded at 12:00 pm and 02:00 pm while minimum densities were recorded at 08:00 am and 06:00 pm. Among honey bee species *Apis mellifera* abundance was significantly higher than other three species. Maximum seed production as well as oil content were obtained from sunflower plots kept under natural conditions, where bee visitors had access to sunflower blossoms. In contrast, sunflower plots covered with insect-proof bags gave minimum

seed production and oil content, which most probably because of bee visitors denied to forage on flowers of the crop. Visitation/foraging of honey bee contributed significantly towards increment in yield, oil content and other quality parameters of sunflower.

TAXONOMY OF SPIDER FROM DISTRICT SOHBATPUR, BALUCHISTAN, PAKISTAN

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Spider fauna of Baluchistan is unexplored, literature review showed very diminutive information about spiders in Baluchistan. Present study designed to fill this gap with special reference to its District Sohbatpur (Located at latitude 28 31' 15'' longitude 68 32' 30''). The District Sohbatpur is rich in agriculture due to Pat feeder Canal. Its main cultivated cash crops are rice, wheat, pea, chick pea, alfalfa, mustards, and oat. These crops have a large number of pests such as aphids, caterpillars, cricket, wire worms, leaf beetles etc are present. Farmers mostly use pesticides to get rid from these pests. But these pesticides have enormous hazardous effect on environment including men. On the other side nature have army of predators which control the pests by biological process which is safe for both fauna and flora. These are Spiders, spiders belongs to order Arachnida, have diverse number of known species (47000 and 114 families) while more waiting for identification. Spiders are voracious, carnivorous and generalist predators and insects are major source of their prey. During present survey 847 specimens were collected in March, 2017 to Dec 2017 and sorted out into 4 families' i.e Lycosidae, Salticidae, Araneidae and Tetragnathae. Among these lycosidae was most abundant with percentage ratio 35.53% (301), Salticidae 29.98% (254) Araneidae 22.31 % (189) and Tetragnathae 12.98 % (103). Collection was done through, Hand picking, Pit fall traps and Net sweeping.

INSECT FAUNA OF FORENSIC IMPORTANCE ON DOG (*CANIS DOMESTICUS*) CARCASSES IN DIR LOWER, KP, PAKISTAN

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Forensic entomology deals with the study of insect biology for the investigations of medico-legal issues. The present study was aimed to evaluate insect fauna of forensic importance on dog (*Canis domesticus*) carcasses in District Dir Lower, KP, Pakistan. The study was carried out in two periods, June 2017 and August 2017. Dogs were sacrificed by killing with shot guns through a veterinary expert and were immediately placed under metallic cages which allowed the access of arthropods to the carcasses and prohibited the access of other carnivores. After collection, the immature stages were preserved in 70 % ethanol while the adult stages were killed in cyanide killing jars and were pinned, labelled and preserved in insect boxes. Insect fauna on the 1st dog carcass consisted of 7 different species belonging to families Calliphoridae, Sarcophagidae, Muscidae, Asilidae (Diptera), Formicidae (Hymenoptera) and Trogidae (Coleoptera). Insect fauna on the 2nd dog carcass consisted of 8 different species belonging to 7 families Calliphoridae, Sarcophagidae, Muscidae, Hippoboscidae, Piophilidae (Diptera), Apidae (Hymenoptera) and Trogidae (Coleoptera). Five distinct stages of decomposition were recognised namely fresh stage, bloated stage, active decay stage, advanced decay stage and dry stage. Mean values of temperature and humidity were recorded in the two study periods which play a key role in the longevity, diversity and succession pattern of insects. The life cycles and succession patterns of insect fauna on dog carcasses can be used to evaluate the post mortem interval (PMI) analysis for medico-legal purposes.

STUDY ON THE PYGMY GRASSHOPPERS (ORTHOPTERA: TETRIGIDAE) FROM SUKKUR DIVISION

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These insects are the smallest representatives of the Orthoptera, size is small less than 20 mm, mainly characterized by having highly elongated, tapered pronotum, that extends over the length of abdomen. Tagmina when present small and scale like and may be exposed or covered by pronotum. Members of the family Tetrigidae (pygmy grasshoppers) may vary in colors, brown grey, rusty grey or moss green in color and can be related to true grasshoppers. Pygmy grasshoppers usually found near water bodies, such as ponds, and streams. Occasionally they also found in dry habitats, woodlands, rice fields and in sandy areas with lichen. These grasshoppers especially eat roots of plants, seedlings, mosses, fungi, algae and cause considerable damage to crops. During the present study 03 species have been reported, pertaining to 02 genera i.e, *Euparatettix* Hancock, 1904, *Ergatettix* Kirby 1914 and species belong to these genera are *Euparatettix indicus*, (Bolivar, 1887) *E.sagittata* (Bolivar, 1887) and *Ergatettix dorsifera* (Walker, 1871). The specimens were collected from grasses, rice fields, plant roots, seedlings and from the boundary of a water pool and the stones with thick mosses growing on them, at the various places of Sukkur division. While two species of genus *Euparatettix*, *E. indicus*, (Bolivar, 1887) and *E.sagittata* (Bolivar, 1887) have been reported for the first time in the region of Sukkur division.

GRASSHOPPER FAUNA OF DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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This study was conducted to know about the grasshopper fauna of district Swabi. Sampling were done from four Tehsils of District Swabi. That were Swabi, Topi, Lahor and Razar. Each Tehsil had three collection points. From Tehsil Swabi collection was done from Shamansor, Anbar, Kunda and famous graveyard of Swabi. From Topi: Jadoon, Bamkhel and Ghazi were the collection site and from Lahor specimens were collected from Manki, Hund and Haryan. From Razar the collection site was Dagi, Yar Hussain and Gohati. The sampling were done regularly two to three times in each month. The sampling timing were at morning. The sampling were done on first and fifteen date of each month. I collected 334 specimens from four Tehsils of District Swabi. Specimens were identified by using key described in Shahid (1964) and we found 28 species, 18 genera, 10 sub-families and 3 families namely as Acrididae, Pygomorphidae and Tetrigidae.

IMPORTANCE OF INSECT POLLINATION IN PAKISTAN

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Pollination is an essential ecosystem service. Effective pollination results in increased crop production, quality improvement and more seed production. Many fruits, vegetables, edible oil crops, stimulant crops and nuts are highly dependent on bee pollination. Worldwide value of pollinators is €153 billion (217 billion US dollars) Value of pollinated dependent crop in Pakistan is 1.59 billion US\$. Of the total value, fruits are dominant with 0.98 billion, vegetables 0.32 billion, nuts 0.15 billion, oil seed 0.13 billion and spices 0.004 billion US \$. Therefore pollination management will be most beneficial for these crops. There are various reasons of pollination deficit such as loss, destruction and degradation of habitats, excessive tillage, destruction of trees, extensive weeding, deforestation,

reduced genetic diversity of nectar plants, pests and pathogens, climate change, extensive and intensive use of pesticides. All these factors individually or in combination are causing the deficit. Some experiments on mango in Multan, Apple in Murree and Brassica in Chakwal indicate that pollination deficit has not yet reached to dangerous levels in these areas. In Pakistan studies on different aspects of pollination has been conducted and bibliography has been produced but still more research is required especially on quantum of deficit in different areas, role of wild bees, rearing of some bees and their utilization in the fields. Academic institutions strengthening in pollination activity is highly needed.

THE IMPACT OF *CEDRUS DEODARA* AND CARBOSULFAN ON THE EMERGENCE OF *TENEbrio MOLITOR* (COLEOPTERA: TENEBRIONIDAE)

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This research was designed to evaluate the survival of mealworm treated with deodar oil and carbosulfan. Mealworms were treated, by feeding method, during larval stage and mortalities were noted in their respective stages. Larvae of 2% were converted into pupae and the rest 98% larvae were unable to pupate on high concentration of deodar oil (12%). On lower dose of 0.75% of deodar oil 46% mean larvae converted into pupae. When larvae were treated with high doses i: e 12% and 6% of deodar oil, no adult emergence was observed. However, the larvae treated with low concentration of 0.75%, 1.5% and 3% concentration of deodar oil, percent emergency was noted as 16%, 7% and 3% respectively. At the highest concentration of carbosulfan (0.5%) no treated larva moulted into pupa. At lower concentration of carbosulfan some of the larvae succeeded to pupal stage. Similarly, the pupal emergence was noted as 10%, 5%, 2% and 1% for 0.03125%, 0.0625%, 0.125% and 0.25% concentrations of carbosulfan respectively. It can be concluded that plant origin oil, deodar oil, may reduce the survival of stored grain pest mealworm.

OVIPOSITIONAL BEHAVIOR OF TETTIGONIIDEA (ENSIFERA: ORTHOPTERA)

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During the present study an attempt has been made to collect the eggs of different species of Tettigoniidea from different habitats. It was noticed that Phaneropterinae mostly laid single egg on the leaves around 16-19 Trigonocorypha unicolor Stoll deposited minimum 2-3 and maximum 8 eggs. It was noticed that species lay their eggs inside the tissues of living plant usually pave the way by first biting a hole with the mandibles and then following up with the ovipositor. Only a single egg is laid in each hole, but in those kinds which exploit the cavity down the center of grass or rush stems a large number may eventually be deposited, filling the center of the stem. In some species the tip of the ovipositor is furnished with series of teeth which assist in sawing directly into plant tissue detail study is in progress.

STUDIES ON THE BIOLOGY, IMMATURE STAGE, SWIMMING AND BURROW EXCAVATING BEHAVIOUR OF *SCHIZODACTYLUS MINOR* (ANDER, 1938) (ORTHOPTERA: SCHIZODACTYLIDAE) FROM SINDH, PAKISTAN

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The present study was carried out on the biology, nymphal stages and life habitats of *Schizodactylus minor* Ander (1938) during 2006-2009 (January to December) on the left bank of river Indus (at Hussainabad, Sehrish

Nagar, and Latifabad. No.4) *S. minor* is a nocturnal and various carnivorous insect, passes through nine nymphal stages, and took one year to complete its developmental period. The detailed description of the various immature stages is given. The burrow habitat and food preference of the cannibalistic species *s. minor* were also investigated swimming activity was also recorded.

SCALING OF FOLIAGE ARTHROPODS OVER TEMPERATURE GRADIENTS AND DIURNAL RHYTHM FROM KINNOW AND VINEYARD ORCHARDS

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Foliage arthropods have profound effects on the vegetation in an area. They play an important role for the pollination of variety of flora. At same time some acts as pest for fruiting trees. Hence, their diversity influenced with the temperature gradients and day and night time. The following research was designed to check the diversity and feeding habits of arthropods in Kinnow and Vineyard orchards. For this purpose, sampling was done from the orchards of nine square in University of Agriculture, Faisalabad. Sampling was done with the help of direct hand picking and sweep net during the session of January-2016 to December-2017. Samples were brought and identified up-to species level in the Biodiversity laboratory. Maximum population was recorded during dawn from Vineyard orchards 47.66% (N=2000). While, 48.99% (N=1847) was recorded from Kinnow orchards. From vineyard at dawn maximum relative abundance 52.34% (N=2196) were observed. From Vineyard, maximum relative abundance of herbivore insects was recorded as 10.75% (N=215) and 11.97 (N=263) as dawn and dusk respectively. While, omnivores and carnivore arthropods were also observed from both sites. After analyzing the (K-S) test, the result showed highly significant result (critical value=0.05, $P \leq 0.001$). it showed that population diversity of both territories was different. As well as the relative abundance of foliage arthropods were different at dawn and dusk times.

BIODIVERSITY OF FOLIAGE INSECTS AND PHOTOPERIOD FROM AGRO-FIELD AND CANAL TERRITORY

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The present work was conducted to find the "Biodiversity of Foliage Insects and Photoperiod from Agro-fields and Canal territory", under ecological conditions of district Okara, Punjab, Pakistan, during the session 2017-2018. "Biodiversity of foliage insects and photoperiod from agro-field and canal territory" throughout the year from February 2017 through January 2018. Total 12 samples were collected from each of the two fields. The composition of insects was documented as: from Agro-fields, total 31 species were found belong to 6 orders, 21 families and 27 genera; while from the Canal territory, total 37 species were found belong to 8 orders, 23 families and 29 genera. From both territories, the total number of insects were found to be 2740 during the entire sampling (12 samples on monthly basis from each territory) and highest abundance was recorded from the Canal territory (N=1430) and least abundance was recorded from Agro-field ie. (N=1310). From monthly based sampling maximum population was recorded from Agro-field, during 1st sampling (180±14.76). Whereas species richness was recorded equal during 2nd, 3rd, 9th and 12th sampling (4 species) at temperature and humidity of 22°C, 34°C, 270°C, 13°C and 21%, 27%, 31% and 58% respectively. Moreover, lowest insects were recorded in 9th sampling (50±5.00). In case of canal territory, highest contribution was recorded during 1st sampling (170±39.12) followed by 160±15.12 (8th sampling). Whereas, species richness was recorded equal during 2nd, 5th (4 species), 8th, 9th (8 species) and 11, 12th sampling with (5 species) at different temperature and humidity. Maximum Diversity (H) was observed from the canal territory (0.46341) and lowest from the Agro-field (0.85717). Evenness was recorded high for agro-field (0.8557) and least for

canal territory as (0.8361). Results from the Shannon Diversity index showed value 2.938 for agro-field and 3.019 for canal territory. Values $P > 0.05$ were non-significant whereas, values ($P < 0.05$) were significant and $P < 0.01$ were highly significant. Variations were recorded owing to the habitat privilege by the occupied taxa.

TICKS (ACARI: IXODIDAE) INFESTATION IN RELATION TO DOMESTICATED ANIMALS IN MULTAN

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Ticks are the main ectoparasites of wild and domesticated animals. These suck the animal's blood and performance of animals decreases due to high tick infestation. Ticks also transmit various contagious diseases like Crimean-Congo Hemorrhagic Fever (CCHF) to animals and humans. For this purpose, the study was carried out to identify ticks fauna of domestic animals in District Multan Punjab, Pakistan at Tehsil level. Ticks were collected from buffaloes, cows, sheep and goats with the help of forceps. The collected specimens were stored in 70% ethyl alcohol. The species were identified under stereomicroscope by using the keys of Hoskins and Walker. A large number of samples were collected from various body parts like ears and tail of the animals. During the study, species from the four genera, *Dermacentor*, *Haemaphysalis*, *Rhipicephalus* and *Hyalomma* were collected. The ticks species belonging to the above-mentioned genera are *Rhipicephalus sanguineus*, *Hyalomma marginatum*, *Hyalomma anatolicum*, *Dermacentor endersoni* and *Dermacentor marginatus*. It has been noted that female and suckling animals have maximum tick infestation than male and old animals. Thus, the study concludes that the females and young ones are more prone to attack of ticks, so an effective strategy should be adopted to tackle with the tick infestation on animals ultimately preventing the spread of disease to human community.

RICE PLANTHOPPERS: POTENTIAL THREAT TO THE SUSTAINABLE RICE PRODUCTION IN THE PUNJAB, PAKISTAN

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The brown planthopper (BPH) and whitebacked planthoppers are considered as major threats to sustainable rice production in Asia. Planthoppers have affected several million hectares of rice area in countries like Vietnam, China, Indonesia, Korea, Japan and Malaysia. In the Punjab which is rice basket for the country is also prone to face the challenge of their ravages which may decrease yield up to 7-10% annually. In case of severe infestation crop fails to yield owing to hopper burn. Studies revealed that average grain yield loss because of planthopper was 10 maunds/ acre, amounting Rs. 15000/ acre during 2017-18. The temporal patterns, IPM strategies and future thrusts are discussed in detail in the paper.

POPULATION DYNAMICS OF MITES ON EGGPLANT IN RELATION TO ABIOTIC FACTORS

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Eggplant (*Solanum melongena* L.) is one of the popular vegetable worldwide and cultivated on large scale in multiple countries such as India, Bangladesh and in Pakistan. Regarding production of brinjal, Pakistan does not rank

at the prominent position. It is because of pest complex most likely the mite fauna in brinjal. Thus, a study is designed to access the population availability of mites. The study was conducted in November 2018, under the field conditions. Data was collected for three times in a week and correlated with the environmental factors (Temperature & Humidity). The results revealed that mite population (Adult and Nymph) was high in the first week of November with 393 individuals / 10 plants and the mite population (Adult and Nymph) was minimum on the third week *i.e.* 55 individuals / 10 plants. In this regard, RH showed negative relation while the temperature showed positive relation. Thus, the study, will help in better understanding of the population fluctuation (mites).

**A NEW SPECIES OF GENUS *ACHETA* F., *A. KARACHIENSIS* (ORTHOPTERA: GRILLIDAE)
WITH SPECIAL REFERENCE TO ITS TEGMEN AND MALE GENITALIA FROM
KARACHI, SINDH, PAKISTAN**

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A new species of *Acheta* F. with reference to its tegmen and male genitalia is described from Karachi, Sindh province, Pakistan. In this paper its male genitalial components and tegmina, are described and compared with its closest ally *A. khanpurensis* Khan and Ahmad from Punjab, Pakistan. Specimens of *Acheta karachiensis* were collected from Karachi, Pakistan, at night by conventional sweeping and hand picking techniques. Preserved them, then the entire specimen was boiled in 10% KOH for few minutes. After washing the specimen the right tegmen was detached and placed on a slide and covered with a cover slip to take the photograph. The genital components. Were dissected out under Nikon SMZ 800 Binocular. The photographs of tegmen and male genitalia were taken by using Nikon Cool Pix 5400 digital camera after placing them under Nikon SMZ 800 Binocular. After completion, the genitalia was preserved in vial with glycerin. Gryllids are generally regarded as important agricultural and domestic pests (Hinton and Cobert, 1949). Ahmad and Khan (2015) redescribed *A. domesticus* (L.) and Khan and Ahmad (2016) described a species *A. khanpurensis* based on male tegmen and genital components. Kamaluddin et al. (2001) and Khan and Kamaluddin (2006) described the external morphology of male and female cricket species *Gryllus bimaculatus* (De Geer) on the basis of their genital components and *Pteronemobius indicus* (Walker) by using its apomorphic characters. Kamaluddin and Khan (2005) described external morphology including tegmen and genitalia of three new species of the genus *Pteronemobius* from Pakistan and also discussed their cladistic relationship.

SPECIES DISTRIBUTION AND RED DATA LIST OF JUMPING SPIDERS FROM PAKISTAN

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Spiders have invaded every habitat on the Earth except polar region, highest mountains and oceans. They play an important role in ecosystem as biological indicator for the changes in habitat quality. Along with that their role in pharmaceutical industries and agroecosystem are under investigation. Among spiders, Family Salticidae is rich in diversity and consists of 6115 species that belong to 636 genera. However, their inventory from Pakistan is incomplete and required attention. Till now only thirty three species of salticid have been reported from Pakistan. Of these, status of nineteen species is dubious. Biogeography and distribution ranges of salticids have never been reported from Pakistan. For this study, a total of 2732 specimens collected from different areas of Pakistan using visual search and netting methods. During surveys, GPS points (longitude, latitude and altitude) were noted for each collected specimen. Bioclimatic profiles were used to assess their potential distribution. BioClim and Maxent model in the Arc GIS were used to predict the potential distribution of the recorded genera of salticids in Pakistan. On the basis of their actual and predicted distribution ranges, 25 recorded species were widespread, four moderately distributed and three restrictively distributed. Among the studied species four were new to science, 23 recorded first time from Pakistan. Species endangered according to IUCN is also recorded from two areas of Pakistan.

TOXIC IMPACT OF IMIDACLOPRID AND LEAD ON DETOXIFYING ENZYMES OF SILKWORM *BOMBYX MORI*

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Silkworms are economically important insect and badly effected by xenobiotics like imidacloprid and heavy metal. They are reared very carefully in the indoor insectaries and mostly exposed to different chemicals through food contamination. In this study, role of detoxifying enzymes in the susceptibility of Imidacloprid (commercial formulation and technical grade) and heavy metal Lead (Pb) were evaluated in 5th instar larvae of *Bombyx mori*. For this purpose larvae were exposed to different concentrations of Imidacloprid and Pb and mortality were recorded after 24h of exposure. The LC₅₀ values of these xenobiotics were estimate and changes in quantity of different enzymes of hemolymph were assessed. The calculated acetylcholinesterase (AChE) in control group and Pb treated group was limited but high in imidacloprid commercial (formulation or technical grade). Silkworm larvae treated with mixture of xenobiotics also have reduce AChE quantity. Catalase (CAT), Glutathione-S-transferase (GST) and Superoxide dismutase (SOD) concentration was high in the mixture of Imidacloprid and Pb as compared to other treatments. The level of these enzymes in Pb treated larvae and control larvae did not differ significantly. Present study demonstrated that detoxifying enzyme such as AChE, SOD, CAT and GST were involved in the free radical scavenging produced in response to xenobiotics exposure in larvae of *B. mori*.

REVISION OF HALYINE SPECIES (HEMIPTERA, PENTATOMIDAE, HALYINI) OF UPPER SINDH, PAKISTAN

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The literature indicates a large number of species have been described to the tribe Halyini from time to time and also place of different species changed from one genus to other by different workers, new records are also explored time to time. During this study, specimens of the tribe Halyini were collected from different districts of Sindh provinces of Pakistan and compared with available literature and preserved material including Holotypes and paratypes and prepared and modified keys of genera and species of tribe Halyini.

EVALUATION OF SILKWORM RACES FED ON LOCAL MULBERRY FOR BIOLOGICAL AND COMMERCIAL PARAMETERS

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The study was conducted to evaluate biological and commercial parameters of three bivoltine races of silkworm i.e. Chinese, Korean and Bulgarian reared in spring 2018 at University of Gujrat. The disease free eggs of Chinese, Korean and Bulgarian races were obtained from Sericulture Research Laboratory, Lahore and maintained at standard conditions i.e. temperature (25 ± 1 °C), RH ($75 \pm 5\%$) and photoperiod (12 light : 12 dark). The mean larval weight (g) of Bulgarian race in 3rd, 4th and 5th instars, 3.14, 4.15, and 6.17 whereas mean larval length (cm) of 2.6800, 3.85, and 5.68 was recorded, respectively. Cocoon weight (1.31 g), cocoon shell weight (0.25 g), cocoon shell percentage (19.087 %) was found in Bulgarian race which was significantly different from other two races reared under same set of environmental conditions. Mean fecundity (436.33), fertility (84.66) and hatchability (80.00 %) were observed in Bulgarian race. The study emphasized on popularizing sericulture in Pakistan by promoting Bulgarian race by conducting more trials and producing hybrids by using other locally available races of silkworms.

SECTION – I V

P A R A S I T O L O G Y

NEW RECORD OF *PSEUDOPHYLLODISTOMUM* (CRIBB 1987) FROM *MYSTUS CAVASIUS* (SILURIFORMES: BAGRIDAE) OF RIVER INDUS SINDH PAKISTAN

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Genus *Pseudophyllodistomum* Cribb, 1987 belong to family Gorgoderidae Loss, 1899. Trematodes belong to this genus are parasites of freshwater fishes. During current studies on helminth parasites of *Mystus cavasius* of River Indus at Jamshoro, Sindh, Pakistan. A total of 17 host fishes were collected from different habitats of study area and brought to the Parasitological Laboratory Department of Zoology, University of Sindh, Jamshoro. During examination of host fishes a total of 7 trematodes belong to genus *Pseudophyllodistomum* Cribb, 1987 was collected, these trematodes have close resemblance with species *Pseudophyllodistomum johnstoni* Cribb, 1987 in all diagnostic features and identified as such. Previously this genus was recorded from freshwater fishes of Australia and Japan, but present species reported from Pakistan. This is first record of genus *Pseudophyllodistomum*, Cribb, 1987 from host *Mystus cavasius* and locality Pakistan.

HISTOLOGICAL STUDY OF BROILER CHICKEN INFECTED WITH MAREK'S DISEASE (MDV)

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In chicken the Marek's disease virus (MDV) is able to cause a variety of syndromes and pathological symptoms. The liver was obtained from broiler chicken in poultry estate, near Gadap, Sindh, the age of birds was 20-25 weeks. The birds had diarrhea, dropping of wings weight loss, anorexia and keel (breastbone) was prominent and protuted out. On postmortem the infected liver was swollen and had lesions, a portion was removed and kept in 10% formalin for 3 days in glass vials and then send to Parasitology Section, Department of Zoology, University of Karachi. The liver tissue was dehydrated in different concentrations of ethanol, and embedded in paraffin wax at 52°C for 4 days, 10 µm sections were stained with haematoxylin and eosin (H & E). The prepared slides were then the observed under the microscope (> × 40) and examined for any histological findings. It was observed that several neoplastic foci pleomorphic cells were beginning to occur and it was infiltrated with lymphomatous lesions consisting of lymphoblast and small to medium sized lymphocytes. These lesions are later transferred into tumour cells and infiltrate different organs and tissues. The study was conducted to assist students of Parasitology and Microbiology in better understanding the pathology caused to liver of chicken (MDV), a highly contagious neoplastic disease caused by alphaherpes virus.

**DESCRIPTION OF NEW TREMATODE *APATEMON HAMALI* N.SP. (TREMATODE:
STRIGEIDAE) IN MALLARD *ANAS PLATYRHYNCHOS* (ANSERIFORMES: ANATIDAE)
OF SINDH, PAKISTAN**

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In result of ongoing helminthological studies of Mallard *Anas platyrhynchos* of Kambar Shahdadt District of Sindh Province, Pakistan, and a total of 22 birds were captured from different localities. During the examination of gut contents and visceral organs, 57 specimens of *Apatemon hamali* n.sp. collected from the intestine of the host bird. *Apatemon hamali* n.sp. differs from its close allies in body shape and size, distribution of vitellaria which is densely scattered in the hind body, the presence of seminal receptacle size and shape of testes and ovary and size of eggs. On the basis of these diagnostic differences, a new species *Apatemon hamali* is proposed. Previously this genus was reported by Bhutta and Khan, 1975 from bird *Oxyura leucocephala* of Pakistan.

**CUTANEOUS LEISHMANIASIS: DISEASE SITUATION IN SOME
DISTRICTS OF SINDH-PAKISTAN**

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Cutaneous Leishmaniasis (CL) is a zoonotic protozoal disease caused by *Leishmania* parasites, which are elongated motile organisms with anterior flagellum. The disease is common in some parts of Pakistan. Present study was under taken to determine the prevalence of Cutaneous Leishmaniasis in districts Hyderabad, Dadu and Jamshoro of Sindh province and its correlation with age, sex, and body parts. A total number of 102, subjects of different age and sex groups were put under investigation. Out of 102 victims, 98 (96.07%) were found positive for Cutaneous Leishmaniasis. The highest Leishmaniasis infection (44.11%) was recorded in 21-30 years age group. Leishmaniasis was higher in males, (65.68%) than in females, (34.31%). The active lesions were more frequent on face (43.13%) followed by hands (30.39%) than other exposed parts of the body.

***HAEMONCHUS CONTORTUS* CRUDE PROTEINS (HCCPS) PRODUCED THE PARTIAL
IMMUNITY AGAINST THE EXPERIMENTAL INFECTION**

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H. contortus is a blood sucking abomasal nematode parasite of small ruminants producing the economic losses. The present study was conducted to explore the immunogenic properties of *H. contortus* crude proteins (HcCP). Protein profile of HcCP was checked by SDS PAGE and immunogenic proteins were recognized by the antiserum produced by using the HcCP as antigen. Infective stage of the *H. contortus* (L3) was used for the challenge infection. Protein band pattern ranging from 10 to 170 was observed and protein bands at 10-20 kDa, 24, 26 and 40-60 kDa were recognized by the antibodies against HcCP through western blot. Faecal egg count reduction test revealed that, at 3rd, 4th and 5th week of immunization egg production was significantly reduced as 75.06%, 76.36% and 57.01 % respectively in vaccinated group as compared to positive control. Impact of the HcCP on Hb level was

also evaluated. After second week of infection significant difference ($P < 0.05$) was found between the negative and positive control. However no significant difference ($P > 0.05$) were found between vaccinated and control groups. Statistically significant difference ($P < 0.05$) was observed among positive control group and vaccinated group at 3rd week of post infection. Packed cell volume (PCV) was also recorded and findings indicated that, no significant differences ($P > 0.05$) was observed among the all groups after the first week of challenge infection. After 2nd week of infection no significant difference ($P > 0.05$) was found among the negative control and vaccinated group, however significant differences ($P < 0.05$) was found between the positive control and vaccinated group. Finally concluded that, HcCPs were able to produce the antibodies, that means these proteins are able to produce the immunity and can be used as vaccine against the infection, however the results of EPG and FECRT indicated that HcCP produced the partial immunity against the infection.

GENUS *HETRAKIS* DUJARDIN 1845 (NEMATODA: HETRAKIDAE) FROM DOMESTIC CHICKEN *GALLUS DOMESTICUS* OF DISTRICT KHAIRPUR SINDH PAKISTAN

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In the current investigation of nematodes parasite in *Gallus domesticus* of district Khairpur, Sindh Pakistan there were 150 chicken were examined for the presence of nematode parasite throughout the examination of gastrointestinal tract of 150 chicken only eight female specimens of genus *Heterakis* were collected from a single host bird. The specimens resemble with *Heterakis gallinarum* Schrank, 1788 in all diagnostic characters like cylindrical body, with lateral cuticular alae; buccal cavity with three circular chops one papillae in each, esophagus cylindrical containing almost round blub at lateral side; nerve ring on the front portion body; valvular aperture at the center of the body and eggs elliptical in appearance and recognize as *Heterakis gallinarum* Schrank, 1788.

THE COMPLETE MITOCHONDRIAL GENOME OF AN AVIAN PLAGIORCHIID AS REPRESENTATIVE OF THE FAMILY PLAGIORCHIIDAE LUHE, 1901

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The Plagiorchiid trematodes have been found in almost all classes of vertebrate hosts, parasitize, the entire digestive tract of their definitive hosts including human. The family Plagiorchiidae is morphologically difficult and controversial family of the superfamily Plagiorchioidea Luhe, 1901. *Plagiorchis* Luhe, 1899 is a type and cosmopolitan genus of the family Plagiorchiidae. Herein, we determined the sequences of *partial larger sub-unit of nuclear ribosomal DNA (28S rDNA)*, *internal transcribed spacers (ITS rDNA)* and *complete mitochondrial genome of Plagiorchis maculosus* Rudolphi 1802 and assessed its phylogenetic relationship with other selected trematodes, based on the mitochondrial (mt) DNA sequences. The sequences results of ITS and 28S rDNA were 100% identical to the corresponding sequences available in GenBank for *P. maculosus*. The complete mitochondrial genome (mtDNA) of *P. maculosus* (14,124 bp) is a circular molecule possessing 36 genes plus a single long non-coding region (NCR) located between tRNA-Gly (G) and *cox3*. The overall A+T content of the entire mt genome, entire 12 PCGs (10,117 bp) and entire NCR (752 bp) is almost same. The NCR contains two sets of tandem

repeats of 283 nucleotides each. The tree obtained from Bayesian inference (BI) *phylogenetic analysis based on concatenated mt protein sequences of P. maculosus with selected trematodes*, indicates that *P. maculosus* has a close genetic relationship with Brachycladiidae and Paragonimidae being classified in the suborder Xiphidiata. The present study described the first mt genome from the type genus of the family Plagiorchiidae and identified the topology that could better explain the position of this family related to other selected trematodes. As species of *Plagiorchis* can parasitize humans, thus the characterization of additional mt genomes is needed from other plagiorchiids for development of mt markers that could help in their identification, phylogenetic and epidemiological studies.

**MITOCHONDRIAL AND NUCLEAR RIBOSOMAL DNA DATASET SUPPORTS THAT
HEPATIARIUS SUDARIKOVI FEIZULLAEV 1961 IS A MEMBER OF THE GENUS
OPISTHORCHIS BLANCHARD, 1895 (DIGenea: OPISTHORCHIIDAE)**

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The taxonomy and classification of the family Opisthorchiidae has been revised by several authors with the exclusion or synonymizing some genera. The genus *Hepatiarius* accommodated two species: *Hepatiarius sudarikovi* Feizullaev 1961 and *H. longissimus* Linstow, 1883. Recently, some experts have invalidated the genus *Hepatiarius* and suppressed as a junior synonym of *Opisthorchis* based on morphological features alone. The molecular data either from nuclear or from mitochondrial DNA is not available from a single species of this genus. In the present study, four specimens of *Hepatiarius sudarikovi* Feizullaev, 1961 syn. of *Opisthorchis sudarikovi*, were recovered from the bile ducts of little egret, *Egretta garzetta*, determined the sequences of complete internal transcribed spacers ribosomal DNA (ITS rDNA) and nearly completed mitochondrial genome and assessed the phylogenetic relationship based on the mitochondrial (mt) DNA sequences. The sequence identity in percent across the ITS rDNA of *O. sudarikovi* were higher, (97.62% in ITS-1 and 95.19% in ITS-2) with that of *Opisthorchis felinus* than other opisthorchiids. The Bayesian inference (BI) *phylogenetic analysis based on concatenated mt protein sequences of O. sudarikovi with selected flukes clustered O. sudarikovi* into the clade of opisthorchiids, with *O. felinus* being the closest related species which supports the affinity of *O. sudarikovi* with trematodes in the genus *Opisthorchis*. This is the first avian liver fluke sequenced for the nearly complete mitochondrial genome. The mt DNA sequences in combination with the rDNA sequences of *O. sudarikovi* provide novel resources of molecular markers for identification, species differentiation and hybridization of *O. sudarikovi* with other avian opisthorchiid flukes.

**NEW HOST RECORD FOR THE GENUS SPIRURA BLANCHARD, 1849, FROM THE INTESTINE
OF PASSER DOMESTICUS LINNAEUS, 1785 (PASSERIDAE: PASSERIFORMES)
IN HYDERABAD, SINDH, PAKISTAN**

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In all 80 House sparrows *Passer domesticus* (Linnaeus, 1785) were collected and investigated for presence of endoparasites from different districts of Sindh Province of Pakistan, out of these 20 examined from Hyderabad, Sindh were found harboring nematodes belonging to genus *Spirura* (02♂, 4♀) in intestine were killed and straightened in

hot 70% ethanol and preserved in ethanol and Glycerol solution for detailed study. Diagrams were made with the help of camera Lucida and photographs were taken with help of Olympus DP-12 digicam. Presently, *Passer domesticus* is a new bird host record for the genus *Spirura* Blanchard, 1849 and the genus is being accredited for the first time from Pakistan.

POLYNEMICOLA INDICUS N.SP. A NEW MONOGEANEAN PARASITE FROM THE GILLS OF FISH POLYNEMUS INDICUS AT KARACHI COAST, PAKISTAN

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There are more than three hundred species of the fish in our sea and at least the same number of monogenetic trematodes. The main objective of present study is to collect and identify the monogenetic trematodes from fishes of Karachi coast. The present monogenean species consist of anteriorly and posteriorly taper elongated body, anterior portion is small and rounded, middle portion narrow while the posterior portion is long and wide. Mouth is ventro-terminal. Two oral suckers are present. Male copulatory organ is with 30 spines arranged in a semicircular fashion in the anterior portion of the body. Testes are six, almost equal in size, situated near the posterior end of the body close to the haptor. Ovary is long, tubular, situated almost in the middle portion of the body. Uterus is long and tubular. Vitellaria consist of numerous small follicles. Haptor is long, to posterior body region irregular. Clamps are numerous, scattered irregularly on the surface of haptor. Clamps consist of (a) marginal sclerite and (b) median sclerites.

TWO SPECIES OF TREMATODE PARASITES IN THE SMALL INTESTINE OF RATS (*Rattus rattus* L.1758) TRAPPED FROM AGRICULTURAL FIELDS OF DISTRICT SWAT, PAKISTAN

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Rats (*Rattus rattus*) were trapped from Rice & Maize fields of district Swat and brought to the VPCI laboratory for investigation of helminth parasitic infection in rat pests. After the autopsy examination of rats, the two species of trematode parasites were recovered from small intestine. The species of the genus *Lutziella* (Rohde, 1966) are described here named *Lutziella swatensis* sp.n and *Lutziella microacetabularae* Rohde, 1966. The new species is characterized by having flat and smooth body, longer than broad, oral sucker with weak musculature, Caeca of irregular shape which bifurcate a little anterior to the ovary. Testes irregularly lobed, cirrus pouch somewhat median, genital pore median a little posterior to the pharynx. Ovary small, post testicular, Laurer's canal present. Uterus filled with eggs occupying most of the body. Eggs small, oval and brown in color. The other species, *L. microacetabularae* is characterized by having long and slender body. Oral sucker subterminal. Esophagus prominent which become gradually wider and bifurcates into two rudimentary caeca. Testes symmetrical, Cirrus pouch median. Genital opening some distance behind pharynx. Ovary submedian, posttesticular uterus occupies most of the hind body. Eggs small, oval, numerous.

PREVALENCE AND ASSOCIATED RISK FACTORS OF BOVINE ANAPLASMOSIS IN RIVER RAVI REGION OF LAHORE PAKISTAN

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Anaplasmosis is a vector-borne, infectious blood parasitic disease of bovine throughout the world. The present study was conducted to investigate the prevalence and associated risk factors of bovine anaplasmosis from the River

Ravi region of Lahore. A total of 1258 bovines (n = 532 buffaloes; n = 726 cattle) were sampled through random sampling strategy and were analyzed for the detection of inclusion bodies resembling anaplasmas through thin smear microscopy. Risk factors regarding specie, breed, sex, age, season and month were analyzed by chi square test in order to check the association of assumed risk factors with the occurrence of disease. A total of 164/1258 [13.04%] bovine were found positive for the occurrence of *Anaplasma*. A slight higher prevalence of *Anaplasma* is reported in cattle 95/726 [13.09%] as compared to buffalo 69/532 [12.97%]. Age, season and months were found significantly associated [$p < 0.05$] risk factors in cattle whereas in buffalo only season and month were found to be significantly associated [$p < 0.05$] risk factors. The bovine population of Lahore is having considerable prevalence of anaplasmosis and risk factors like age, season and month were found to be statistically associated with the occurrence of disease.

PARASITIC INFESTATION IN GULFAM FISH (*CYPRINUS CARPIO*) CARP FROM KEENJHAR LAKE DISTRICT THATTA SINDH, PAKISTAN

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Study has been carried out six month period from January 2017 to June 2017. The parasitic infestation in different fish species at Keenjhar lake District Thatta. with special reference to Gulfam (*Cyprinus carpio*) carp fish, Among the 78 fishes of family carp were examined for parasitic infestation. The present study observed the parasitic prevalence in Gulfam fish (*Cyprinus carpio*) carp fish the 46 fishes species of Gulfam (*Cyprinus carpio*) species were collected. The 18 fishes species of silver carp (*Hypophthalmichthys*), 14 fishes species of Rahu (*Labeo rohita*) and 14 fish species of Morakhi (*Cirrhinus mrigala*) 14 were examined for parasitic infestations during the study period. The highest infection rate (25.6%) was observed in Gulfam (*Cyprinus carpio*) followed by (11%) was recorded from Morakhi (*Cirrhinus mrigala*) and 5% Rahu (*Labeo rohita*). Silver carp (*Hypophthalmichthys*). The present study showed that the fish species were infected by the fishes were infected by ectoparasites they were studied four different species of parasites were found in these fishes *Trichodina* sp, *Ergasilus* sp, *Argulus* and *Lernia cyprinus*. The fish were found to be infected with species of parasites while mixed infections were far less *Cyprinus carpio* found to be the most prone. Parasitic infection increases with increase in temperature the diversity of parasites was more pronounced than their frequency or number in this area Keenjhar lake Thatta Sindh Pakistan.

NEW LOCALITY RECORD OF GENUS *LABRIFEROIDES* (TREMATODA: FAMILY LEPOCREADIIDAE NICOLL, 1934) COLLECTED FROM FRESHWATER CLOWN KNIFE FISH *CHITALA CHITALA* (HAMILTON, 1822) OF RIVER INDUS AT SUKKUR, SINDH, PAKISTAN

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In continuation of the research project no. 20-3426 on Biodiversity of helminth parasites of freshwater fishes of river Indus in Sindh province of Pakistan, a total of 12 freshwater Clown Knife Fish *Chitala chitala* (Hamilton, 1822) were collected from the river Indus at Sukkur, Sindh, Pakistan. Helminthological examination of different parts of Clown Knifefishes exposed only single trematode of genus *Labriferoides* (Trematoda: family Lepocreadiidae Nicoll, 1934) collected from the intestine of the hosts. This genus is being recorded for the first time from Pakistan and from host Clown Knife Fish *Chitala chitala* (Hamilton, 1822), thus making it new host and locality record.

**NEW RECORD OF GENUS *TANAISIA* SKRJABIN, 1924 IN *VANELLUS LEUCURUS*
(CHARADRIIFORMES: CHARADRIIDAE) OF HYDERABAD, SINDH, PAKISTAN**

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During present study on abundance of helminth parasites of *vanellus leucurus*, only one trematode of genus *Tanaisia* was collected from the kidney of the host. It has flattened, cylindrical, muscular body tapered anteriorly and rounded posteriorly; cuticle spines present; oral sucker round; prepharynx absent; pharynx muscular, overlapped by oral sucker; intestinal ceca smooth, running in lateral field of body, partially overlapped by uterine eggs and vitellaria united posteriorly forming cyclocoel; ventral sucker absent; testes two, juxtaposed; ovary median, intercecal, pretesticular, irregular in outline, located in anterior part of second quarter of body; seminal receptacle present, immediately anterior to ovary; uterus filled with eggs, yellowish, dark brown in color, filling entire body. On the basis of these diagnostic characteristics, the present specimen has close resemblance with *T. dubia* Freitas, 1951 and identified as such. This genus is being reported for the first time from the host *Vanellus leucurus* in Pakistan, making it a new host record.

NEW HOST RECORD FOR GENUS *PHILOMETRA* FROM FRESH WATER GARFISH *XENENTODON CANCELLA* (CAMALLANIDA: PHILOMETRIDAE) OF RIVER INDUS, SINDH, PAKISTAN

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In continuation of the research project no. 20-3426 on Biodiversity of helminth parasites of freshwater fishes of river Indus in Sindh province of Pakistan, a total of six freshwater garfishes *Xenentodon cancella* (Hamilton 1822) were collected from the river Indus at Kotri, Sindh, Pakistan. During examination of gut contents and visceral organs two nematodes were collected from the body cavity of the two hosts. On the basis of morphological features one nematode was identified and placed in genus *Philometra*. This genus is previously reported from the Karachi, Pakistan from *Eleutheronema tetradactylum*. This genus is being recorded for the first time from the host garfish *Xenentodon cancella*.

***HOLOSTEPHANUS SINDHENSIS* SP.N. (TREMATODA: CYATHOCOTYLIDAE) POCHE, 1926
FROM *MILVUS MIGRANS* (BLACK KITE) IN LARKANA SINDH, PAKISTAN**

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During survey of Helminth Parasitic infection from *Milvus migrans* (Black Kite) were purchased from the District, Larkana, Sindh Pakistan. The birds were autopsied in the Parasitological laboratory for collection of internal Helminth parasites, fifteen hosts were examined in random intervals, out of which twelve were found infected with twenty five trematodes, these specimens were recovered from small intestine. The specimens were mounted permanently according to standard procedure. A detail study was conducted and a new species *Holostephanus sindhensis* sp.n. is proposed. The new species is characterized by having: The body of the specimens is oblong almost spherical with narrow anterior and broader posterior end. Although the ventral cavity is not obvious but it appears that it is present, covering almost whole body of the specimens. Oral sucker terminal, oval in shape followed by very

short pharynx. Ventral sucker small, rounded situated above the starting of vitelline follicles. Hold fast organ is long, elongated appears vertically located like kidney shaped, covers greater part of the body and occupies anterior and posterior half of the body with in the ventral cavity, slightly displaced to the left side. Pre-pharynx absent. Esophagus and intestinal caeca are mostly obscured by vitelline follicles. The testes are tandem situated in anterior half of the body. They are rounded or spherical and unequal in size. The anterior testis is larger in size slightly attached by the hold fast organ. Posterior testis is smaller in size. Posterior testis lie besides the hold fast organ, both testes are approximately same in size. Cirrus pouch short, situated in the centre of the body and extend from about the posterior of the hold fast organ. Genital opening situated in the posterior most broader line of mid body region. Ovary is spherical and post-equatorial in position, displaced to the right side. The vitellaria are extensively developed, composed of large follicles, which are distributed laterally, extend from behind the ventral sucker reaches up to the level of posterior of the hind body. Uterus short, eggs containing varying from 2-6 in numbers, double walled, oval to rounded in shape. As the present specimens do not match exactly with already recorded species, it is reported to be new as *Holostephanus sindhensis* sp.n. The genus and species are first record from District Larkana, Sindh, Pakistan.

***EPISTHMIUM* ALYKHANI SP.N. (TREMATODA: ECHINOSTOMATIDAE LOOSS, 1899) FROM THE
BUBULCUS IBIS (CATTLE EGRET) IN LARKANA, SINDH, PAKISTAN**

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Parasitic infection is a serious health problem in the world, especially in developing countries. This study was conducted to find the Helminth parasites in *Bubulcus ibis* (Cattle egret) from District Larkana, Sindh, Pakistan. Ten birds were dissected, out of ten, seven were found infected. A total sixteen trematodes were collected. The trematodes were fixed, preserved, dehydrated, stained and finally permanently mounted in Canada balsam for further detailed study and identified as belonging to the family Echinostomatidae Looss, 1899, genus *Episthmium* Luhe, 1909 was recovered in small intestine of Cattle egret and proposed as new species *Episthmium alykhanis* sp.n. The new species is characterized by having: Body of specimens is flattened, long, aspinose, leaf shaped. Maximum width attained at acetabular level. Tegumental spines start from below the pharynx up to the acetabular region. Head collar well developed it bears 18 spines of which are on each lobe arranged in single row, not interrupted dorsally. Lateral spines are larger in size. Oral sucker is sub-terminal, rounded and much smaller than ventral sucker. Pre-pharynx absent, Pharynx well developed. Intestinal bifurcation start from the pharynx reaches up to the end of hind body. Ventral sucker is rounded in shape situated in middle of the body. Testes have smooth outline, tandem, unequal in size occupy greater part of posterior region of the body. Anterior testis is cup shaped smaller in length and larger in width from posterior testis. Posterior testis is roughly spherical shaped. Ovary pre-testicular, horizontal elongated occupy in cup or slightly above in the cup of anterior testis. Cirrus sac is large, pouch shaped with narrower anterior and broader posterior slightly overlaps by ventral sucker. Genital opening lie between intestinal bifurcation and above the acetabulum. Vitellaria dense commence from below the bifurcation arranged in lateral fields, reaches up to the anterior region of posterior testis. Uterus more occupy between cirrus pouch and ovary. Eggs are oval shaped, double walled. The Species name is in honour of Dr. Aly Khan a renowned Parasitologist in Pakistan.

**TREMATODES OF GENUS *PLEUROGENOIDES* (LUHE, 1901) TRAVASSOS, 1921 (DIGenea:
PLEUROGENIDAE) FROM FRESHWATER FISH *Ailia coila* (HAMILTON, 1822) OF
RIVER INDUS, SINDH, PAKISTAN**

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In continuation of the research project no. 20-3426 on Biodiversity of helminth parasites of freshwater fishes of river Indus in Sindh province of Pakistan, a total of 38 freshwater fishes *Ailia coila* (Hamilton, 1822) were collected from several locations of the river Indus, Sindh, Pakistan. The gut contents revealed the presence of 143 trematodes

belonging to genus *Pleurogenoides* (Luhe, 1901) Travassos, 1921. All hosts *Ailia coila* were found to be infected with trematodes of this genus. Previously this genus is recorded from the frogs of Pakistan. The presence of genus *Pleurogenoides* in freshwater fish is reported for the first time from Pakistan.

**TREMATODE OF GENUS *ECHINOCHASMUS* DIETZ, 1909 (TREMATODA: ECHINOSTOMATIDAE)
COLLECTED FROM FRESHWATER FISH *CATLA CATLA* (HAMILTON, 1822)
OF RIVER INDUS, SINDH, PAKISTAN**

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In continuation of the research project no. 20-3426 on Biodiversity of helminth parasites of freshwater fishes of river Indus in Sindh province of Pakistan, a total of 11 freshwater fish *Catla catla* (Hamilton, 1822) were collected from the river Indus at Jamshoro, Sindh, Pakistan. The gut contents revealed the presence of the trematode of genus *Echinochasmus* Dietz, 1909. The genus *Echinochasmus* Dietz, 1909 contains a large number of species that are transmitted to human as food borne trematode infection. This is first record of genus *Echinochasmus* Dietz, 1909 from *Catla catla* of Sindh province, Pakistan.

***TETROCHETUS CORYPHAENAE* (DIGenea: ACCACOELIIDAE) FROM THE
DOLPHINFISH *CORYPHAENA HIPPURUS* (PERCIFORMES: CORYPHAENIDAE) OF
GWADAR COAST, BALUCHISTAN**

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Coryphaena is a genus of marine ray-finned fishes, the only known genus in its family. Dolphinfishes are some of the fastest-growing species in the ocean being cultured in developed countries and can reach up to about 88 pounds weight. During laboratory investigations on the helminth parasites of Dolphinfish *Coryphaena hippurus*, a total of 45 trematodes belonging to genus *Tetrochetus* were recovered from the intestine of 4 host fishes. On the basis of morphological features, the present specimens have close resemblance with *T. coryphaenae*. Previously there is no record of *T. coryphaenae* from Pakistan as well as from the host fish *Coryphaena hippurus*, therefore, making it new host and locality record.

**PREVALENCE OF HELMINTH PARASITES IN *RATTUS NORVEGICUS* (RODENTIA: MURIDAE)
IN JAMSHORO, SINDH, PAKISTAN**

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In continuation of the research project no. 9412-NRPU on assessment of parasitic diversity in Rats and Mice (Rodentia: Muridae) of human habitations in Hyderabad Division, Sindh, Pakistan, a total of 9 hosts *Rattus norvegicus* were randomly collected from the Jamshoro city. The helminthological examination of gut contents and visceral organ revealed highest prevalence for the nematodes (69%), followed by trematodes (44%) and cestodes (39%). None of the acanthocephalan was recorded.

HELMINTH PARASITIC INFECTIONS OF DISTRICTS HYDERABAD, JAMSHORO AND NAUSHARO FEROZE SINDH, PAKISTAN

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Study on avian helminth parasites are important both from economic and zoonotic point of view. Comparatively very less research has been conducted on Helminth parasitic infection of common avian in Pakistan, especially none from the district Nausharo Feroze of Sindh Province. Collected specimens belong to four classes i.e. Cestoda, Trematoda, Acanthocephala and Nematoda. A total of twenty-one different species were found during present investigation. Out of twenty-one, three species are from class Cestoda, fourteen species are from class Trematoda, one species belongs to Acanthocephala and three species are from Nematoda. The birds procured were *Himantopus himantopus*, *Vanellus indicus*, *Ardeola grayii*, *Columba livia*, *Zenaida macroura*, *Acrido thestristis*, *Passer domesticus*, *Gallus gallus domesticus*, *Coturnix coturnix* and *Centropus sinensis*. The helminth parasites were processed according to the standard procedures for detail study. The Helminth (Endo) parasites recovered are: CESTODES: *Cotugnia fleari* (Meggit, 1927) Bilquees, 1985; *Raillietina (R.) galeritae* (Skrjabin, 1914) Khan and Habibullah, 1967; *Choanotaenia gondwana* (Inamdar, 1934) Khan and Habibullah, 1967. TREMATODES: *Uvitellina megacaecatum* sp.n.; *Cotylurus cornutus* (Rudolphi, 1808) Szidat, 1928; Bhutta and Khan, 1975; *Apatemon* Szidat, 198; *Echinostoma rafiae* sp.n.; *E. garzetti* sp.n.; *Episthmium jamshorensis* sp.n.; *E. sindhensis* sp.n.; *E. biqeesae* sp.n.; *Heterotestophyes heckmanni* sp.n.; *H. Gibsoni* sp.n.; *H. jonesae* sp.n.; *Knipowitschiatrema pakistanensis* sp.n.; *K. sternuli* sp.n.; *Stictodora alykhani* sp.n. ACANTHOCEPHALA: *Centrorhynchus cribbi* sp.n. NEMATODES: *Subulura* Molin, 1860; *Cyrnea columbi* sp.n.; *Diplotriana* Railliet et Henry, 1909.

INITIAL RESULTS OF AN ASSESSMENT STUDY ON NEMATODE DIVERSITY IN FRESH WATER FISHES OF THE RIVER INDUS AT SINDH, PAKISTAN

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During present study 331 fishes belonging to 14 species of fresh water fishes were collected from February 2017 to November 2017; they were brought either alive or preserved in Department of Zoology, Sindh University Jamshoro. They were dissected as per method and organs were removed and placed in normal saline and teased gently for dislodging nematodes. The collected nematodes were cleared and preserved in 70% alcohol and glycerine solution. The temporary slides of some specimens were made for identification. Out of 331 examined fishes 25.07% were infected with nematodes. The highest prevalence was noted *Aorichthys aor* (55.55%) whereas *Cirrhinus* were negative. The collected nematodes belong to genus *Camallanus*, *Paracamallanus*, *Falcaustra*, *contracaecum*, *Goezia* and *Monhysterides*. Furthermore, present research has high academic and commercial value from perspective of methodology and content of knowledge. It will set precedent of parasitic study of fresh water fauna.

STUDIES ON THE LUMEN DWELLING HELMINTHS AND PROTOZOANS INFECTION OF HUMAN IN KARACHI AND THEIR PREVALENCE

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The current study was aimed to find the frequency of human intestinal parasites in Karachi hospitals and medical centres and to know as to which age group was most prone to infection. Human intestinal parasitic infections are a serious health issue of public in developing countries of world which affect around 3.5 billion people directly and indirectly (Noori *et al.*, 2016). The causes of human intestinal parasitic protozoal and helminthic infections are improper hygiene, lack of access to safe drinking water and poor sanitation. They can lead to chronic diseases such as iron deficiency anemia, stunted growth children, physical and mental health problems, cognitive impairment, diarrheal diseases, malnutrition, protein depletion, vitamin deficiencies, intestinal obstruction and increased susceptibility to other infections. In this retrospective cross-sectional study of stool samples from patients with gastrointestinal symptoms were collected from January 2015 to October 2016 from five hospitals of Karachi. Out of 2212 samples 274 (12.4%) were positive. The most prevalent was *Entamoeba histolytica* (66.1%) followed by *Giardia lamblia* (22.3%), *Ascaris lumbricoides* (5.5%), *Blastocystis hominis* (1.8%), *Hymenolepis nana* (1.8%), *Entamoeba coli* (1.1%), *Iodamoeba butschlii* (0.7%), *Ancylostoma duodenale* (0.4%) and *Taenia saginata* (0.4%). Children and teenager twenty or < 20 years were the most infected. *Entamoeba histolytica* and *Giardia lamblia* are the most prevalent protozoan and *Ascaris lumbricoides* and *Hymenolepis nana* are most common helminths in Karachi.

SPONTANEOUS FRACTURE OF LONG BONE BY *ECHINOCOCCUS* SP. – A CASE REPORT

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Hydatid cyst is an occasional source of sudden death, with only a few cases being reported in the clinical literature. The purpose of this study is to present a rare case of hydatid infection in woman complaining pain in thighs and suspecting osteomyelitis/tuberculosis, the X-ray revealed that the patient had cystic lesion. The removed lesion was sent to Aga Khan Laboratory for histopathological evaluation where on microscopy it was found to be hydatid cysts of bone of various sizes. Adjacent to the tear, bone tissue presented in homogeneous necrotic areas, situated on generalized dystrophic background.

NEW RECORD OF A NEMATODE RECOVERED FROM *FRANCOLINUS PONDICERIANUS* (GALLIFORMES: PHASIANIDAE: PERDICINAE) FROM SINDH PAKISTAN

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Francolins are also known as small partridge or spur fowls, belonging to family Phasianidae (subfamily Percinidae) of galliform birds. During present study 15 birds of grey partridge *Francolinus pondicerianus* were captured from different localities of Sindh province and examined for their helminths parasites. Out of 15 only three birds were found infected with

24 specimens of nematodes. These nematodes were preserved in 70% ethanol and glycerin solution. After the identification only two specimens were belonged to the genus *Subulura* Molin, 1860. Previously, genus *Subulura* with different species was reported in the world from different hosts but not reported from grey partridges, therefore at present, it is the first record of this genus from the body cavity of *Francolinus pondicerianus*, making new host record from Sindh, Pakistan.

PREVALENCE OF METAZOAN PARASITES IN SNEAKHEAD FISH, *CHANNA STRIATA* (ANABANTIFORMES: CHANNIDAE) IN SINDH, PAKISTAN

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In the present study, total of 18 specimens of snakehead fish, *Channa striata* were collected from different areas of district Sanghar, Sindh, Pakistan during September 2016 to January 2018. These fishes were brought in Advanced Parasitology Research Laboratory, Department of Zoology, University of Sindh, Jamshoro. About 13 fishes out of 18 were found infected with different metazoan parasites. These metazoan parasites were included nematodes and acanthocephalan in helminth parasite and fish lice and gill parasites in ectoparasites. Prevalence of these parasitic varieties in *Channa striata* was recorded as 4% by nematodes of genus *Paracamellanus*, 6% by fish louse of genus *Argulus*, 28% by acanthocephalan of genus *Pellisentus* and 62% by gill parasites of genus *Lamproglana*. The highest parasitic burden was recorded 72.22% by ectoparasites, while the parasitic burden of endoparasites was recorded 11.11% in the present host fish.

TREMATODE FAUNA OF MIGRATORY GAME BIRDS FROM DIFFERENT WATER BODIES OF SINDH, PAKISTAN

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The game bird industry is a significant industry in our country, used as a source of meat proteins, eggs, etc. they are considered as a valuable source of earning in many areas of Sindh. Different birds, particularly Hilal ones are trapped from different freshwater lakes. In the present study, the total 114 birds of family Anatidae of order Anseriformes were collected including common pochard (*Aythya ferina* L.), tufted duck (*Aythya fuligula* L.), white eyed pochard (*Aythya nyroca* Guldenstadt), green-winged teal (*Anas crecca* L.), shoveler (*Anas clypeata* L.) and Mallard (*Anas platyrhynchos* L.); during the years 2017 to 2018 from different water bodies of Sindh, Pakistan. Birds were brought in the laboratory, the dead birds were dissected according to the standard procedure of dissections; their organs were examined for trematodes under stereo microscope. The research showed out of 114 birds, 97 were found positive with 85% prevalence of infection by trematodes of seven species. These species were identified as *Paramonostomum aythiae* Thebo *et al.*, 2018, *Psilochasmus fuligulae* new species, *Psilochasmus oxyurus* (Creplin, 1825), *Catatropis pakistanensis* Schuster and Wibbelt 2012, *Echinochasmus amphibolus* Kotlan, 1922, *Notocotylus attenuatus* (Rudolphi, 1809), *Echinostoma revolutum* (Frolich, 1802) and *Cotylurus cornutus* (Rudolphi, 1808). This is the first detailed study on the parasitic forms of migratory game birds, mainly waterfowls in Sindh, Pakistan. Therefore, the findings in present research will be a new addition in parasitological faunistic studies generally and in helminthology particularly.

A BASELINE SURVEY OF THE ENDOPARASITES IN THE LOCAL CHICKEN *GALLUS GALLUS DOMESTICUS* (LINNAEUS, 1758) OF SINDH, PAKISTAN

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Chicken *Gallus gallus domesticus* Linnaeus 1758 are important and most commonly found domestic fowl. Chickens are omnivorous and are the mostly eaten food in Sindh Pakistan. Chickens are susceptible to different

parasites including ticks, lice, fleas, mites and intestinal worms. Since, chickens are important source of food in Sindh, it is farmed at large scale. Therefore, the studies on chickens need to focus on its parasitic aspects. In this regards, a baseline survey of the endoparasites was held in the month of March 2018 in Karachi, Thatta and Sujawal districts of Sindh and a total number of 64 domestic chickens was sacrificed and the endoparasites were obtained from the digestive tract of the chickens. Yet 19 chickens were found parasite positive. While as the identification of parasites is still ongoing and it has found that the nematode which have been identified are *Capillaria spp.*, while as the found Cestodes are *Raillientina tetragona*, and *Hymenolepis*. Further studies are continued to discover the all possible internal parasites.

NEW SPECIES OF THE GENUS *COLOCERAS* TASCHENBERG, 1882 (PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) FROM *STREPTOPELIA SENEGALENSIS* (COLUMBIFORMES: COULMBIDAE) FROM SINDH, PAKISTAN

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Laughing dove, *Streptopelia senegalensis* (Columbiformes: Columbidae) is a resident bird of Sindh, these are found throughout Africa, the Middle East, some parts of Asia and Australia. During the present investigation, fifteen birds were examined for their chewing lice, collected from different regions of lower Sindh. The collected specimens of the chewing lice were identified as genus *Coloceras*, were mounted permanently in Canada balsam and examined under light microscope for its species identification, measurements, drawing and chaetotaxy. The specimens were compared with the different species of genus *Coloceras* and identified as new species. The taxonomic diagnostic differences appeared between chaetotaxy, morphometry, male and female terminalia and male genitalia. The name of new species has been proposed as *Coloceras dharejo* sp.n. in the honour of eminent parasitologist of the country, Professor, Dr. Ali Murtaza Dharejo to recognize his huge contribution in the field of parasitology.

PREVALENCE OF HELMINTH PARASITES IN COMMON TEAL, *ANAS CRECCA* L. (ANSERIFORMES: ANATIDAE) FROM DISTRICTS THATTA AND JAMSHORO, SINDH, PAKISTAN

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The present study constructed on the quantitative parasitological parameters used to determine the prevalence of helminth parasites in Common Teal, *Anas crecca* collected from the two localities, Manchar Lake of District Jamshoro and Keenjhar Lake of District Thatta, Sindh, Pakistan. During the period of 10 months from August, 2017 to May, 2018, a total of 100 common teal birds were examined for the helminth parasites from both localities. A variety of helminth parasites was recovered from the intestine, liver, gizzard and body cavity of these birds with 63% over all prevalence. Among helminthes, the highest prevalence was reported by nematodes 89%, following by the infection of cestodes and trematodes with 46% and 18% respectively. However, no acanthocephalan were recovered from common teal.

INVESTIGATION OF TWO MIGRATORY BIRDS, *FULICA ATRA* AND *GALLINULA CHLOROPUS* (RALLIDAE: GRUIFORMES) FOR CHEWING LICE INFESTATION FROM SINDH, PAKISTAN

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Fulica atra (Eurasian Coot) and *Gallinula chloropus* (Common moorhen) belong to Family Rallidae of Order Gruiformes. Coots are commonly available in Sindh province and considered palatable by the people of this

province. Total 100 birds were examined in which 80 were found positive with 80% prevalence. In the present study, coots and moorhens have been observed to be infested by five genera of three families Menoponidae and Laemobothriidae of Suborder Amblycera and Family Philopteridae of suborder Ischnocera. Total more than 2500 specimens were recovered, comprising of seven species till present, including *Fulicoffula lurida* (Nitzsch, 1818), *Pseudomenopon pilosum* (Scopoli, 1763), and *Pseudomenopon lanceolatum*, (Tendeiro, 1955) with new host and *Incidifrons fulicatrae* sp.n., *Pseudomenopon sindhiensis* sp.n. as new species to science; species of the genus *Rallicola guimareesi* (Emerson, 1955) and *Laemobothrion atrum* (Nitzsch, 1818) as new locality records from Pakistan.

A NEW RECORD AND A NEW SPECIES OF FEATHER MITE, *DERMONOTON SINDHIENSIS* SP.N. (ACARI: SARCOPTIFORMES: PTEROLICHOIDEA: KRAMERELLIDAE) FROM SINDH, PAKISTAN

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A new species of the feather mite of genus *Dermonoton* Guad and Mouchet, 1959 was introduced in the present study, which has been recovered from Laughing Dove, *Streptopelia senegalensis*. This genus was reported for the first time from Sindh, Pakistan. There were total 12000 specimens of mites were recovered from 157 birds, birds were collected from district Thatta, Jamshoro, Hyderabad and Badin, Sindh, Pakistan during February 2017 to March 2018. The mites specimens were mounted permanently in Hoyer's medium and were identified as new species, *Dermonoton sindhiensis* sp.n. The present species of mite was studied taxonomically and compared with its closely related species in having general body shape and size, prosoma, opisthosoma and genital pores of male and female bodies, the sclerotization, chaetotaxy and striations of the dorsal carapace of the body which varies in all related species, on the basis of which a new species is proposed with the specific epithet on its type locality.

PREVALENCE OF ECTOPARASITES IN LARGE RUMINANT ON PRIVATE FARMS OF CHOLISTAN DESERT, BAHAWALPUR

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Ectoparasites are the common problem for domestic animals. The present study was conducted to determine the prevalence of ectoparasite in domestic buffaloes and cattle in the private farms of Cholistan desert, Bahawalpur. A total of 250 hosts were examined during the present study. The overall prevalence was found (41/250) 16.4%. The ectoparasites like ticks and lice were collected from the hosts. Buffaloes and cattle were found mixed infection but the lice showed dominant (n=27) prevalence as compared to ticks (n=14), this prevalence showed significant results (P<0.05). Out of 250 buffaloes and cattle, 35 were males and 215 females. The prevalence of ectoparasite showed non-significant (P>0.05) results in males 9(35.71%) as compared to female 32(14.88%). The prevalence showed significant results in age group (P<0.05) and highest prevalence occurred in 3-4 years old age group. Among 250 hosts 150 were cows and 100 were buffaloes. Which 30/150(20%) cows were infected and 11/100 (11%) buffaloes were infected prevalence showed significant (P<0.05) results between two species, but the values apparently showed that the prevalence of ectoparasites in cows was higher than in buffaloes. In conclusion the lice were more prevalent as compared to ticks in cattle of local farmers of Cholistan and gender showed greater prevalence in males than females of both hosts. Buffaloes were more immune than cows. Age of the hosts have significant influence on the ectoparasites.

POPULATION DENSITIES OF SOUTHERN ROOT KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) AFFECT GROWTH AND YIELD OF CUCUMBER

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In the present work a new species of *Acheta* F. is described from Karachi, Pakistan with special reference to its sound producing apparatus and song patterns with comparison the common house cricket *Acheta domesticus* (L.) and recently known species *A. khanpurensis* Khan and Ahmad 2016 found from Pakistan. The morphology of stridulatory file, stridulatory teeth, plectrum, number of teeth, pattern of song, and its frequency were observed to identify the new species. The stridulatory apparatus are important characters in the classification of Ensifera (Desutter-Grandcolas, 2002). Ensiferen acoustic evolution is determined on stridulation organs in Gryllidae (Alexander, 1962; Bailey, 1991; Otte, 1992). Stridulation is considered as a main character to decide its species level. *Acheta* sp. was collected from different areas of Karachi Pakistan at night. Male songs were recorded with the help of Handy recorder. Then recorded, songs were sliced with the help of Audacity 1.3 Beta (Unicoded) software. These sounds were studied and analyzed by using Matlab software. After song recording the specimen was boiled for few minutes to soften the body and detached its right tegmen. Placed the tegmen on stub from its ventral surface. Then the stub was put into a desiccators with Silica gel to dry it. Tegmen was coated with auto coater into JOEL model No. JFC-1500 Japan with gold target, which coated up to 300° A and scanned with Scanning Electron Microscopy, JOEL Japan Model No. JSM- 6380 A, from Centralized Science Laboratory, University of Karachi, Karachi. Then studied the SEM pictures of the file.

PATHOGENIC AND REPRODUCTIVE POTENTIAL OF *MELOIDOGYNE INCOGNITA* ON FIVE CHILI 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.

PREVALENCE OF *HAEMONCHUS CONTORTUS* IN GOATS AND SHEEP AT HILLY REGIONS OF BEHA MATTA DISTRICT SWAT, KPK PAKISTAN

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Haemonchus contortus is the natural helminth parasite of small ruminants around the world. This helminth is a leading source of death and sickness by affecting health and breeding capacity of animals. Loss of weight, failure to

grow well, anemia, hypo-proteinemia, bottle jaw, diarrhea and lethargies are the principal symptoms of the disease. Present study was designed to find the prevalence of *H. contortus* and its effect on the blood of sheep and goats in hilly areas of Swat, Pakistan. For this purpose the stool & blood samples were collected from the sheep and goats. The fecal samples were kept in 10% formaldehyde & then transported into the research laboratory of Parasitology, Department of Zoology, University of Malakand. Each of the samples was diagnosed microscopically through Wet Mount Techniques. This study was conducted from January to December, 2016. Out of the total 300 animals including (n=150) sheep and (n=150) goats were screened for *H. contortus* infection. The overall prevalence was 26.6 % (n=80/300) including 15.6 % (n=47/300) sheep and 11 % (n=33/300) goat was recorded. Regarding age of the ruminants, the highest prevalence 54% (n=27/50) in < 9 months sheep than > 9 months 20% (n=20/100). Likewise in goats higher prevalence 36% (n=18/50) was noted in ruminant < 9 months than > 9 months 15% (n=15/100). In general the infections frequency in both sheep and goat was greater in ruminants having age \leq 9 months 15% (n=45/300) than in ruminants \geq 9 months 11.67% (n=35/300). In the fecal sample analysis for disease pattern in sheep showed a higher rate of prevalence in females 10% (n=30/300) than males 5.66% (n=17/300). Similarly, the fecal sample of goats' positive sample was higher in females 7.66% (n=23/300) than males 3.33% (n=10/300). Based on the findings of present research it was concluded that sheep and goats is the important sector for economic development of a country. This sector should be treated regularly for the infectious diseases agents through modern technologies.

PREVALENCE OF INTESTINAL PARASITIC INFECTION AMONG FOOD HANDLERS IN UNIVERSITY OF MALAKAND KHYBER-PAKHTUNKHWA PAKISTAN

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Present study was aimed to assess the prevalence of intestinal parasitic infection among food handlers in University campus lower Dir, Khyber Pakhtunkhwa Pakistan. A total of 535 respondents were enrolled, out of the total 535 were agreed to give stool samples while 45 were found medically unfit. The data was collected thorough standard procedures and techniques. The stool samples collected were brought to Laboratory of Parasitology, department of Zoology for further studies. Of the total food handlers examined, 59.8 % (320/535) were found to be infected with one or more intestinal parasites. The percentage of prevalence among food handlers inside the University was 28.9% (155/535) and that of outside the University was 30.8% (165/535). The prevalence of mono-parasitism 48.5% (260/535) among them 47.6% (255/535) are infected with *helminths* parasite, and one individual 0.93% (5/535) is infected with intestinal protozoan (*Entameba histolytica*). The prevalence of poly parasitism is that, 8.4% (45/535) were infected with two different parasitic species, among them, 6.5% (35/535) were infected with two helminthic parasite, and 1.86% (10/535) are infected with one protozoan and one helminthic parasite). Fifteen individuals 2.8% (15/535) are infected with three parasitic species, two helminths and one protozoan intestinal parasite. The study shows that IPIs are still an important public health problem in Pakistan. Therefore we need to educate the people especially food handlers, would be the best way to prevent transfer of IPIs.

NEMATODE FAUNA OF DISTRICT BAJORE, KPK, PAKISTAN

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Nematodes are the most abundant and diversified group in the animal kingdom. These are second only to insects in the number of species in the animal kingdom. Their distribution extends to particularly all conceivable habitats where any other life forms could barely exist; they nematodes are bound to be present. The systematic

studies carried out so far on plant parasitic, free-living marine, soil nematodes and insect parasitic nematodes resulted in a total of 50 samples, out of these the following nematodes are found during the current survey. *Meloidogyne javanica*, *Tylenchus*, *Filenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Psilenchus*, *Criconemoides*, *Aphelenchus*, *Xiphinema*, *Longidorus*. From soil nematodes *Ironus*, *Acrobeles*, *Mesorhabditis*, *Alaimus*, *Acrobeloides*, *Cephalobus* and *Mylonchulus* are found from above mentioned areas. Some entomopathogenic nematodes also found viz., *Steinernema pakistanense*, *S. litorale* and *Heterorhabditis indica*. These nematodes are first time reported from bajore district.

ENTOMOPATHOGENIC NEMATODES FROM LAWAT DISTRICT NEELUM, AZAD JAMU KASHMIR

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Lawat is situated in Neelum Valley District Neelum. Neelum Valley is 144 km long bow-shaped deeply forested region in Azad Kashmir, Pakistan. Using *Galleria mellonella* "soil trap" method, a survey for entomopathogenic nematodes was conducted during 2018. Two species of *Steinernema* were found. Out of these one new record species i.e., *S. kandi* and other known species *S. litorale*. In the present study deals with the description, morphology, morphometrics molecular studies and efficacy against different insect pests as a biopesticide. Both species are first time reported from Lawat district Neelum, Azad jamu Kashmir.

EPIDEMIOLOGY AND RISK FACTORS ANALYSIS OF FASCIOSIS IN BUFFALO POPULATION OF DISTRICT BAGH, AZAD KASHMIR, PAKISTAN

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This study was carried out in district Bagh of Azad Kashmir from December, 2016 to May, 2017. During this short term investigation fecal sample from 200 buffaloes (*Babylus bubalis*) from 11 different study sites were collected, it was observed that 80 (40%) animals out of 200 have parasites in their body. The presence of *Fasciola hepatica* was determined in these collected samples by using the floating and sedimentation technique. Liver samples of slaughtered buffaloes were also examined during study period. Liver samples were taken from freshly slaughtered buffaloes and made the cuts for assessment of fasciola presence by the maceration process. It was found that 74 samples have Fasciolosis out of total 200 samples, so 37% per cent samples contain *F. hepatica* and 63 per cent slaughtered buffaloes samples were negative. These results indicates that some animals of the area have fasciola species in their body, which is resulting economic losses to the owners, in the form of less production of milk and meat; and more fatalities among the livestock and causes economical losses to farmers. During this study overall prevalence rate of fasciolosis has been found 19.25%. This study provided the percentage of presence of fasciola in fecal as well as in liver samples.

THE SURVEY ON THE ECOLOGICAL ROLE IN THE IMMUNOLOGY OF THE ECTOPARASITES OF ARDEA CINEREA OF SINDH, PAKISTAN

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Parasites are the potent selective force driving both the distribution and abundance of host species (Grenfell and Dobson, 1995). Ectoparasites are the external obligate parasites which are found practically on the outer body parts

of animals and play their distinguished role in the life of the birds. Unfortunately the ecological and immunological aspect of avian ectoparasites is not studied in Pakistan yet. Ecological immunology is the emerging research field which focuses to explain the variation in the immune function across individuals, populations and species. The ectoparasites and their bird hosts are mostly used in the measurements of the cost of immune function in relation to the life cycles. Studies on the ectoparasites are though found in Pakistan but, not a clear study is done on the ecological aspect of the immunology of ectoparasites of birds to determine the host-parasite interactions in terms of the effect of environment. In this study, grey heron (*Area cinerea*) was selected for study. The grey heron has a slow flight, with its long neck retracted. Fishes, amphibians and small insects is its diet. According to central European grey herons host 29 species of parasitic worms like *Apharyngostrigea cornu*, *Posthodiplostomum cuticola*, *Echinochasmus beleocephalus*, *Uroproctepisthmium bursicola*, *Neogryporhynchus cheilancristrotus*, *Desmidocercella numidica*, and *Bilharziella polonica* (Sitko, J.; Heneberg, P. 2015). Total number of 10 birds *Ardea cinerea* from the different regions of Sindh have been collected and the ectoparasites are detected from the outer body parts. Finally the ectoparasites have been sent for further studies.

EVALUATION OF THE EFFICACIES OF ALBENDAZOLE AND MEBENDAZOLE AGAINST ANCYLOSTOMIASIS IN SCHOOL GOING CHILDREN OF DISTRICT SWAT, KP, PAKISTAN

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Soil transmitted helminths are amongst the negligible tropical diseases mainly occur in under developed countries of the World. Current trail was pin pointed to record the efficacies of albendazole and mebendazole against ancylostomiasis as well as occurrence of intestinal parasitosis amongst school children of district Swat, KP, Pakistan. At the start of the study, the section heads of the schools were requested for consent. For parasitological analysis, faecal samples were obtained from each student for diagnosing. These faecal samples were preserved in 10% formalin, and carried out to Laboratory of Parasitology, Department of Zoology, University of Malakand, where they were examined through light microscope by using flotation method, direct smear method in Lugol's solution and usual salt solution. Out of total 64.8% (n=192/296) were found infected with *Ancylostoma duodenale*. Total no of infected individuals lost were noted 18.7 % (n=36/192). Present study revealed light 87.8% (n=137/156) moderate 10.8 % (n=17/156) and heavy 1.2 % (n=2/156) intensity of infection. 1st grade students were found highly infected as 86.2% than the respective grade of students. Only height and weight were noted significant (p<0.05). Albendazole show high rate of efficacy 75% than mebendazole 71% (p>0.05). Present study concluded that: (1) Albendazole was showing high rate of efficacy than mebendazole and (2) soil, raw vegetables and contact with pet animals are the most common risk factors analysed. Further studies are required to understand the efficiencies of various anti-helminthic drugs and risk factors associated are suggested for the future studies.

PREVALENCE AND RISK FACTORS OF ASCARIS LUMBRICOIDES AMONG SCHOOL CHILDREN'S IN LOWER DIR, PAKISTAN

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To investigate the prevalence and risk factors of *A. lumbricoides* infection among school children's in Lower Dir district, Pakistan. A cross sectional study was made among 387 (grade 1 to grade 5 of 6 to 14 years in age) school

children. A structured questionnaire was used for information collection. Of the total examined subjects 60% (192/320) were found infected with *A. lumbricoides*. The children with low ages were highly infected than the children of advanced classes as 1st grade 25.5%, height 40-47, 41.6%, weight 14-27kg 39%, and upper arm circumference 5-6". Socio economic characteristics resulted that children's with illiterate father 72.3% , animal keepers 86.9% , use of raw vegetables 65.1%, water source other than tube well 53.6%, individuals do not wash their hands were more infected than those who wash hand after defecation 58.3% , children who were suffer previously from gastrointestinal complaint were more infected 57.2 % then those who were not infected. In total 81.2% children's were infected light, 17% moderate and 1.5% were heavily infected comparing with WHO recommended values. Present study gives new insight to school aged children's infection status with *A. lumbricoides*. This study concludes that drinking water other than tube well, use of raw vegetables and no hand washing before eating and after defecation were the leading risk factors associated for the transmission of *A. lumbricoides* in the population of the study area. The results underscore the need for planning prevention and treatment strategies for the infection.

EVALUATION OF THE EFFICACIES OF ALBENDAZOLE AND MEBENDAZOLE AGAINST ANCYLOSTOMIASIS IN SCHOOL GOING CHILDREN'S OF DISTRICT SWAT, KP, PAKISTAN

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Soil transmitted helminths are amongst the negligible tropical diseases mainly occur in under developed countries of the World. Current trail was pin pointed to record the efficacies of albendazole and mebendazole against ancylostomiasis as well as occurrence of intestinal parasitosis amongst school children of district Swat, KP, Pakistan. At the start of the study, the section heads of the schools were requested for consent. For parasitological analysis, faecal samples were obtained from each student for diagnosing. These faecal samples were preserved in 10% formalin, and carried out to Laboratory of Parasitology, Department of Zoology, University of Malakand, where they were examined through light microscope by using flotation method, direct smear method in Lugol's solution and usual salt solution. Out of total 64.8% (n=192/296) were found infected with *Ancylostoma duodenale*. Total no of infected individuals lost were noted 18.7 % (n=36/192). Present study revealed light 87.8% (n=137/156) moderate 10.8 % (n=17/156) and heavy 1.2 % (n=2/156) intensity of infection. 1st grade students were found highly infected as 86.2% than the respective grade of students. Only height and weight were noted significant (p<0.05). Albendazole show high rate of efficacy 75% than mebendazole 71% (p>0.05). Present study concluded that: (1) Albendazole was showing high rate of efficacy than mebendazole and (2) soil, raw vegetables and contact with pet animals are the most common risk factors analysed. Further studies are required to understand the efficiencies of various anti-helminthic drugs and risk factors associated are suggested for the future studies.

STUDIES ON HAEMATOLOGICAL VARIATIONS IN FRESH WATER FISH *CHANNA PUNCTATUS* (BLOCH, 1793) INFECTED WITH TREMATODE PARASITES

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Present study was conducted to determine the haematological parameters in channa punctatus (Bloch) naturally infected with trematode parasites. For the purpose, 200 channa punctatus with average body weight of 335±18.8 g were examined. For the hematological investigations, haemoglobin concentration, red blood cells count, white blood

cells count, platelets, lymphocytes counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated. Total erythrocyte count mean values of non-parasitized and parasitized fishes noted. There is a significant change in erythrocytes count, haemoglobin concentration and hematocrit values. While the total leukocyte count was increase in the parasitized as compare to the non-parasitized channa punctatus. This study concluded that the infected fish showing several changes in hematological parameters that may often cause anaemia.

ASSESSMENT OF THE PARASITISM POTENTIAL OF THREE PARASITOIDS OF FRUIT FLY, *BACTROCERA* SPP. (DIPTERA: TEPHRITIDAE) UNDER LABORATORY CONDITIONS

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Parasitism potential of pupal parasitoid, *Dirhinus giffardii* and larval-pupal parasitoids, *Diachasmimorpha longicaudata* and *Aganapis daci* was assessed against *Bactrocera* spp. under laboratory conditions. Three different kinds of hosts viz. *B. zonata*, *B. dorsalis* and *B. cucurbitae* were reared on artificial larval diet and known number of pupae and larvae of each fruit fly species were offered to the parasitoids in glass cages in a no choice test (Pupae to *D. giffardii* and larvae to *D. longicaudata* and *A. daci*). Results on the parasitism potential of *D. giffardii* on pupae of different fruit fly species revealed that the highest per female parasitism was recorded on *B. zonata* (16.72 ± 1.67). Adult emergence percentage of *D. giffardii* did not differ significantly among all the three *Bactrocera* spp. Sex ratio of the emerged parasitoids revealed maximum percent females (61.64 ± 2.67) from pupae of *B. cucurbitae*. Similarly per female parasitism by *D. longicaudata* was also significantly the highest on larvae of *B. zonata* (26.40 ± 1.79) with maximum adult emergence percentage of 93.41 ± 2.54 . Sex ratio of the emerged *D. longicaudata* did not differ significantly. Parasitism rate of *A. daci* was significantly the highest on larvae of *B. zonata* (24.88 ± 2.01) with insignificant differences in the adult emergence percentage. Sex ratio of *A. daci* showed that percent females from the emerged parasitoids were the highest (54.2 ± 3.44) on *B. dorsalis*. Relative collective parasitism per female by all the three fruit fly parasitoids revealed that highest parasitism rate (23.7 ± 2.26) was exhibited by *D. longicaudata* followed by *A. daci* (22.72 ± 2.14). The study manifested *B. zonata* as the ideal host for laboratory rearing of all the three parasitoids.

INCIDENCE OF GASTRO INTESTINAL PARASITES FOUND IN LARGE RUMINANTS IN DISTRICT LOWER DIR KHYBER PAKHTUNKHWA PAKISTAN

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The study was conducted to determine the prevalence of gastrointestinal (GI) parasites in cattle and Buffalos of Lower Dir Khyber Pakhtunkhwa Pakistan. The presence of helminth eggs, protozoan cysts and oocysts in case of sporozoa in fecal samples were detected using direct smear methods and concentration techniques which include floatation, centrifugation and sedimentation. Identification of eggs cyst and oocysts was done on the basis of differential morphological features. Out of 314 cows and buffaloes examined, 85.59% were found positive for eggs of one or more species of GI parasites. The prevalence of parasitic infection was higher in buffaloes (63.55%) as compared to cows (75.61%) but the difference was non-significant ($p > 0.05$). Sex wise prevalence of GI parasites was higher in female as compared to male ($p > 0.05$). Prevalence in young cow was 53.65% (22/41) and adult cow 56.12% (87/155) as well as, in young buffalo the occurrence was found young buffalo 38.23% (13/34) while in adult buffalo 73.8% (62/84), however, age wise differences was also non-significant ($p > 0.05$). Prevalence in spring cow was 32.39% (23/71) and summer cow 68.8% (86/125) as well as, in spring buffalo the occurrence was found 52.94%

(27/51) while in summer buffalo 71.64% (48/67). Gastro-intestinal parasites are serious issues in cattle and buffaloes of district Lower Dir Khyber Pakhtunkhwa, Pakistan. The prevalence rate of GI parasite with season wise more in summer as compared to winter. Also the burden of parasitic infection was low in most animals warranting treatment.

PREVALENCE OF *ENTEROBIUS VERMICULARIS* IN SCHOOL CHILDREN OF MALAKAND REGION, THE NORTHWESTERN PART OF PAKISTAN

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We investigated the prevalence of *Enterobius vermicularis* in school children of four districts in Malakand region, Pakistan. A total of 400 stool samples were examined from May 2014 to July 2017 using direct smear (Normal saline and Lugol's Iodine solution) the concentration methods and procedures. Twenty three (5.75%) individuals were found infected with *E.vermicularis* ovum. Five (1.25%) were infected with only *E.vermicularis* and eighteen (4.5%) with other helminths. *E.vermicularis* 23(5.75%), hook worm 11(2.75%), *Ascaris lumbricoides* 5 (1.25%), *Taenia saginata* 2 (0.5%) and *Trichuris trichiura* 4 (1%) were detected. Age wise 5-8 years were more parasitized followed by 13-15 and 9-12 years of age. Male children were highly infected than females. Children in Malakand district were found more infected followed by Dir Upper. Similar infection rate was noted in children of districts Dir Lower and Swat. *Enterobius vermicularis* is commonly parasitizes children. These type of studies should be conducted time to time to know the hazardous of parasitosis for the betterment of the human health.

PREVALENCE OF *RHABDOCHONIASIS* IN SNOW TROUT OF RIVER SWAT AND RIVER PANJKORA, KHYBER PAKHTUNKHWA PROVINCE, PAKISTAN

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Fish provides primary source of high quality protein to more than one billion people in the world. This research was aimed to explore the helminth parasitic diseases in *Schizothorax plagiostomus* (the snow trout) from river Swat and river Panjkora, Pakistan. A total of 360 fish specimens were collected from the lower, middle and upper reaches of both the rivers through gill nets, cast nets, dragon nets and hooks. All the specimens were examined in the Laboratory of Parasitology, Department of Zoology, University of Malakand for ecto and endo- parasites during the months from January 2015 to December 2018. Of the total examined fish samples 21.9% (n=79) were infected with *R.schizothoracis* including 17.7% (n=32/180) in river Swat and 26.6% (n=47/180) in river Panjkora. Highest month-wise prevalence ($p=0.9878, p<0.05$) was recorded in the month of May (30%), followed by February and October each (26.6%) while the lowest during August (13.3%). Highest prevalence ($p=0.9723, p<0.05$) was reported in summer season (26.6%), while lowest in the winter season (20%). Adults were highly infected ($p=< 0.0001$) in prevalence (63.7%) followed by sub-adults (13.2%) while no infection was found in juvenile specimens. Female fish samples had higher ($p= 0.0277, p>0.05$) prevalence (28.8%) than males (16.6%). Fishes of the lower reaches had highest ($p=0.0029, P>0.05$) prevalence (31.7%) followed by middle reaches (16.5%) while the lowest rate of prevalence was noted in the fish samples collected from upper reaches (9.87%). Present study address that *R.schizothoracis* in the intestine of snow trout has a long term relationship and call as a natural infection in cyprinids and zoonotic risk to human.

OCCURRENCE OF SOME NEMATODE PARASITES IN THE GASTROINTESTINAL TRACT OF ARIIDAE (TELEOSTEI: SILURIFORMES) CATFISH, ARIUS DUSSUMIERI (HAMILTON, 1822) FROM KARACHI COAST

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Current study was carried out in November, 2017 to investigate the occurrence of helminth parasites in catfish, *Arius dussumieri* (Valenciennes, 1840) belonging to family Ariidae (Bleeker, 1862). Thirty specimens of *Arius dussumieri* (Valenciennes, 1840) off the Karachi coast were examined for the occurrence of helminth parasites. Fish were examined after washing contents of gastrointestinal tract and observed under light microscope with the help of regular parasitological methods. The nematode parasites namely; *Raphidascaris acus* (Bloch, 1779) larvae, *Metabronema magnum* (Taylor, 1925) and *Haplonema immutatum* (Ward et Magath, 1917) were recorded from the gut of the catfish, *Arius dussumieri* (Hamilton, 1822). These are new host records as these parasites have not been reported from *Arius dussumieri* species in the region off the Karachi coast.

IN VITRO ANTILEISHMANIAL EVALUATION OF CRUDE ETHANOLIC EXTRACTS FROM SELECTED MEDICINAL PLANTS

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In the present study, nine selected traditional medicinal plants were collected from different areas of Matta and Kabal Tehsils of District Swat, Khyber Pakhtunkhwa. The selected collected plants were *Debreageasia salicifolia*, *Tagetes minuta*, *Berberis lycium*, *Ajuga bracteosa*, *Aesculus indica*, *Calotropis procera*, *Chenopodium botrys*, *Daphne oleoides* and *Salix babylonica*. The ethanolic crude extracts of these plants were evaluated *in vitro* for evaluating the efficacy of these extracts against the promastigotes of *Leishmania tropica*. Five different concentrations of each plant extract and one negative control were prepared and were placed in two 96-well microtitre plates containing 1×10^5 *L. tropica* Promastigotes/well. The stock concentration of the extracts was 1000 µg/mL which was used in 1000 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL, and 50 µg/mL concentrations. The 96-well plates were incubated at 26°C for 72 hours and the number of *Leishmania tropica* promastigotes in all the wells was counted microscopically by using an Improved Neubauer Haemocytometer. Luckily the crude extracts of the plants *Berberis lycium* eliminated majority of the promastigotes of *L. tropica*, while the remaining 8 plant extracts also showed promising antileishmanial activity. Fifty percent inhibitory concentration (IC₅₀) of all the extracts as evaluated in Graph-Pad Prism 6 Software for all the nine the used plants after 24, 48 and 72 hours respectively were 1.337 µg/mL, 1.363 µg/mL, and 1.400 µg/mL for *Berberis lycium*; 1.293 µg/mL, 1.350 µg/mL, and 1.353 µg/mL for *Tagetes minuta*; 1.299 µg/mL, 1.315 µg/mL, and 1.367 µg/mL for *Aesculus indica*; 1.287 µg/mL, 1.323 µg/mL, and 1.349 µg/mL for *Chenopodium botrys*; 1.277 µg/mL, 1.321 µg/mL, and 1.361 µg/mL for *Ajuga bracteosa*; 1.279 µg/mL, 1.316 µg/mL, and 1.346 µg/mL for *Calotropis procera*; 1.236 µg/mL, 1.318 µg/mL, and 1.344 µg/mL for *Daphne oleoides*; 1.260 µg/mL, 1.291 µg/mL, and 1.345 µg/mL for *Salix babylonica*; and 1.212 µg/mL, 1.273 µg/mL, and 1.368 µg/mL for *Debreageasia salicifolia* crude ethanolic extracts. Our results indicate that these selected plant extracts, their fractions and compounds could be valuable in the designing and development of new anti-leishmanial drugs.

GENETIC CHARACTERIZATION OF *PARAMPHISTOMA SP.* THROUGH ITS REGION SEQUENCING ANALYSIS, FROM DISTRICT MARDAN, PAKISTAN

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Paramphistomosis is the most prevalent disease of wild and domestic animals, causing heavy economic losses in many countries across the world. The morphological identification of these parasites is very difficult, molecular characterization is used to discriminate different species of *Paramphistoma*. Present study was conducted to identify genetically *Paramphistoma spp* of district Mardan. All the samples of these rumen flukes were collected from buffalos, no parasite was found from other animals e.g. cattle, sheep and goat. DNA was isolated from fully mature flukes and ITS region was amplified for sequence analysis. Comparison of partial sequencing of ITS region showed that 4 flukes were 100% similar that there was no intraspecific variation. Alignment results of ITS sequences of present study with reference sequencing available in GeneBank (NCBI) showed that these flukes were *Paramphistoma cervi* and 95% similarity with the flukes reported from China. This was the first study in which *Paramphistoma species* were identified genetically from District Mardan.

GASTRO-INTESTINAL PARASITIC INFECTION IN SMALL RUMINANTS OF UPPER DIR DISTRICT, KHYBER PAKHTUNKHWA, PAKISTAN

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Prevalence of gastrointestinal parasites in small ruminants of District Upper Dir Khyber Pakhtunkhwa Pakistan were investigated. A total of 312 (n=184 sheep and n= 128 goats) faecal samples were collected. Overall prevalence of parasite in sheep and goats were 94.0% (n=173/184) and 86.7% (n=111/128) respectively. The parasite species found in sheep were: *Strongyloides spp* (41.30%), *Haemonchus contortous* (21, 73%), *Trichuris spp* (17.39%), *Faciola spp* (13.58%). Adults were more infected by *Haemonchus contortous* as compared to young ones. In adults' sheep the prevalence of *Ascaris spp* was: 40.90%, *Haemunchus contortous* 31.81% and *Trichuris spp* 27.27%. In young the prevalence was 42.5%, 32.5% 25.0% respectively. In adult goats the prevalence of *Ascaris spp* was: 38.70%, *Haeamunchus contortous* 32.25%, *Trichuris spp* 19.2% and in young the prevalence was 35.7%, 32.1%, 28.5% respectively. Higher infection of *Haemunchus contortous* in male as compared to female, prevalence in male sheep was: 47.82% (88/184) and female sheep 46.19% (85/184) as well as, in male goats the prevalence was found 39.06% (50/128) while in female goats 47.65% (61/128). The intestinal parasite incidence was significantly greater in monsoon than summer and winter season. As prevalence in spring sheep was 31.52% (58/184) and summer goats 61.82% (115/184) as well as, in spring goats the prevalence was found 29.68% (38/128) while in summer goats 57.03% (73/128). In risk factor grazing system, prevalence in grazing sheep was 45.10% (83/184) and stalled fed goats 48.91% (90/184) as well as, in grazing goats the prevalence of parasites were found 55.46% (71/128) while in stall fed goats 31.25% (40/128). In risk factor treatment, prevalence in treated sheep were 26.08% (84/184) and untreated goats 67.93% (125/184) as well as, in treated goats the prevalence was found 25.00% (32/128) while in untreated goats 61.71% (79/128). The correlation of intestinal parasite prevalence with pregnancy status and age was not found. It is concluded that prevalence of *Haemonchus contortous* in sheep was higher than goats, due to variance in hygienic surroundings of the both hosts.

GASTROINTESTINAL NEMATODES OF THE BUKHARA DEER: THEIR IDENTIFICATION AND PREVALENCE

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The Bukhara deer or Bactrian deer (*Cervus elaphus bactrianus* Lydekker, 1900) named as Xonguli is the rare and unique species of the original deer species, spread over the Amudarya river valleys in the desert of Central Asia. The Bukhara deer is a severely endangered species, its total population having fallen to just 360 by 1998 -1999 years. The numbers at the reserve have been steadily recovering to reintroduce the species into the Zaravshan river valley. Of course the deer are not confined to the reserve but migrate in and out of the forest. The purpose of the study was to investigate the species composition of helminthes and the dynamics of infection of Bukhara deers using parasite eggs and larvae in Uzbekistan. In this study fecal samples were collected and examined from different years old Bukhara deers which were protecting in nature reserves of Zarafshan and Badai tukai in Uzbekistan. In the helminthological study of Bukhara deers, their age, the level of infection and the number of eggs per one gram of fecal were highlighted. DNA was extracted using a DNA Tissue Kit (Macherey-Nagel, Germany). Were investigated partial sequences of the internal transcribed spacer (ITS) rDNA. Sanger sequencing from both ends was performed by the "Genome" center (Moscow, Russia). As a result of the morphological observation, the nematodes belonging to the genus of *Haemonchus*, *Marshallagia*, *Ostertagia*, *Teladorsagia* and *Nematodirus* were discovered in Bukhara deers. As a result of molecular research, we were able to detect *Marshallagia marshalli*, *Haemonchus contortus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta* and *Nematodirus spathiger* species. These nematodes were parasitic in the gastrointestinal tract, and their rate of infection was 22.4%, and the average number of eggs was 11-44 copies. It should be noted that it was the first attempt in Uzbekistan in this area. Using molecular genetics method, it is possible to implement the most effective and accurate diagnosis for animal's helminthiasis during their lifetime, and may be helpful in future use as a species-specific marker in the PCR-diagnostic system for relevant veterinary laboratories.

MOLECULAR CHARACTERISTICS OF TWO SPECIES OF THE GENUS OF MARSHALLAGIA (NEMATODE: OSTERTAGIINAE)

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Nematodes of the genus of *Marshallagia* (Orloff, 1933) - parasites of abomasum and fine intestine of ruminants. They have a significant impact on the metabolism of host animals and can cause significant economic damage. According to our data the species of *Marshallagia marshalli* Ransom, 1907 is widespread in Uzbekistan and the detection rate ranks first among the nematode of this genus. In our studies we used ITS sequences of rDNA species of *Marshallagia marshalli* and *Marshallagia dentispicularis* Assadov, 1954 parasites of the abomasums and small intestines of sheep (*Ovis aries*). The material collected during autopsies at abomasum of sheep to slaughterhouses on the farms of Bukhara and Kashkadarya regions of Uzbekistan. ITS-1 + 5.8S + ITS-2 ribosomal DNA fragments were obtained in GeneAmp PCR System 2700 thermal cycler and using primers: 18Sd71:5'-GTCCCTGCCCTTTGTACACACCGCCCG-3'; 28Sr2: 3'-TGGTAGCCGTTTCTCAGGCT-5'; 18SQ39: 3'-GAAACCTTGTTACGACTTTTRCBYGG-5' и 28Sd1: 5'-ACCCGCTGAAYTTAAGCATAT-3'. The PCR products were sequenced from both directions with the primers used in the amplification by the center of "Genome" using an Applied Biosystems 3730 DNA Analyzer automatic sequencer. According to the results of research obtained and compared from each test sample and nematode *M. marshalli* and *M. dentispicularis* fragments of ITS-1+5.8S+ITS-2 length of about 2,000 base pairs (KT428384). In this case the variable regions observed in ITS-2, and the remaining portions of sequences were virtually identical. Samples *M. marshalli* differed from *M. dentispicularis*

in two nucleotides. The percentage was 0.2%. The observed difference is noted as intraspecific. The remaining fragments were identical. The high degree of similarity regions ITS-1 + 5.8S + ITS-2 confirms the assumption that the species *M. dentispicularis* and *M. marshalli* are not independent. We believe that these species can be attributed to a single species - *M. marshalli*. Next, we investigated the difference *M. marshalli* and representatives of other taxa Ostertagiinae. This difference in ITS-2 between *M. marshalli* and *Ostertagia ostertagi* were significantly higher and amounted to 8.3%. Thus, a comparative study of samples of DNA of one species of nematodes from different regions and by different owners - revealed a degree of intraspecific variability. A comparative study of DNA samples of nematodes of the same species from different regions and from different hosts will reveal the degree of intraspecific variability, and in the applied aspect will make it possible to use it as an additional diagnostic method using molecular studies to determine the taxonomic identity of parasitic nematodes.

GASTROINTESTINAL NEMATODES OF THE BUKHARA DEER: THEIR IDENTIFICATION AND PREVALENCE

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The Bukhara deer or Bactrian deer (*Cervus elaphus bactrianus* Lydekker, 1900) named as Xonguli is the rare and unique species of the original deer species, spread over the Amudarya river valleys in the desert of Central Asia. The Bukhara deer is a severely endangered species, its total population having fallen to just 360 by 1998 -1999 years. The numbers at the reserve have been steadily recovering to reintroduce the species into the Zaravshan river valley. Of course the deer are not confined to the reserve but migrate in and out of the forest. The purpose of the study was to investigate the species composition of helminthes and the dynamics of infection of Bukhara deers using parasite eggs and larvae in Uzbekistan. In this study fecal samples were collected and examined from different years old Bukhara deers which were protecting in nature reserves of Zarafshan and Badai tukai in Uzbekistan. In the helminthological study of Bukhara deers, their age, the level of infection and the number of eggs per one gram of fecal were highlighted. DNA was extracted using a DNA Tissue Kit (Macherey-Nagel, Germany). Were investigated partial sequences of the internal transcribed spacer (ITS) rDNA. Sanger sequencing from both ends was performed by the "Genome" center (Moscow, Russia). As a result of the morphological observation, the nematodes belonging to the genus of *Haemonchus*, *Marshallagia*, *Ostertagia*, *Teladorsagia* and *Nematodirus* were discovered in Bukhara deers. As a result of molecular research, we were able to detect *Marshallagia marshalli*, *Haemonchus contortus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta* and *Nematodirus spathiger* species. These nematodes were parasitic in the gastrointestinal tract, and their rate of infection was 22.4%, and the average number of eggs was 11-44 copies. It should be noted that it was the first attempt in Uzbekistan in this area. Using molecular genetics method, it is possible to implement the most effective and accurate diagnosis for animal's helminthiasis during their lifetime, and may be helpful in future use as a species-specific marker in the PCR-diagnostic system for relevant veterinary laboratories.

EPIDEMIOLOGICAL AND CLINICAL PROFILE OF CUTANEOUS LEISHMANIASIS IN PATIENTS ATTENDED AT DIVISIONAL HEADQUARTER TEACHING HOSPITAL, MIRPUR AJ&K

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Cutaneous leishmaniasis is a vector borne protozoan disease that is endemic in 98 countries with an incidence of 1.5 to 2 million infections each year. The present study was conducted on epidemiological and clinical profile of cutaneous leishmaniasis in patients attended at Divisional Head Quarter Teaching Hospital, Mirpur Azad Jammu &

Kashmir from January 2017 to March 2018. During the entire study, a total of 73 patients were suspected for cutaneous leishmaniasis, among them 43 were smear positive for amastigotes giving an overall prevalence of 0.19% in patients attended at dermatology clinic of DHQ Hospital Mirpur, AJ&K. The disease was higher in adults (58.14%) as compared to young ones (41.86%). Male and female patients accounted for 58.14% and 41.86% of infections, respectively. Cutaneous leishmaniasis was unevenly distributed in rural (62.79%) and urban (37.21%) population. The disease was more common in winter (53.49%) as compared to other seasons. Wet lesions were more frequently (72.09%) reported than dry lesions (27.91%). The patients presented with single and multiple lesions were 86.05% and 13.95%, respectively. The travel history, contact with poultry and pet animals were not associated cutaneous leishmaniasis. A population based study, including the possible reservoirs, will increase our understanding and possible control of disease persisting in Mirpur Division, Azad Jammu & Kashmir.

PARASITES AND HUMAN EVOLUTION

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Modern humans started their journey to far-flung areas of the world from Africa approximately 100,000 years ago. They settled in areas where they had to adapt to challenging and unfamiliar climates, find different ways to feed themselves and fight off new pathogens. Modern studies suggest that it was the pathogens, particularly parasitic worms, that had the biggest role in driving natural selection but that genetic adaptation to them may also have made humans more susceptible to autoimmune diseases. This presentation will help explain the impact of parasites on the evolution of human genome.

ECTOPARASITE INCIDENCE IN CHILTAN WILD GOAT (*ARTIODACTYLA: CAPRINAE*) INDIGENOUS OF HAZARGANJI CHILTAN NATIONAL PARK (HCNP), BALOCHISTAN, PAKISTAN

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The ectoparasite study is of great importance to monitor its effect on the health and care of wild goat population. A study was carried out to determine the *ectoparasites* of chiltan wild goat (*Capra aegagrus chiltanensis* Lydekker, 1913) in Hazarganji chiltan national park (HCNP) between the months of May to October, 2016. Hard ticks (Ixodidae) species were found in HCNP ground area where high mobility of chiltan goats were observed. It can be assumed in the light of literature that fully or partly fed ticks might cast off from chiltan wild goats. The prevalent ectoparasites in this study were from the two genera of tick viz *Hyalomma*, and *Rhipicepalus* (*Boophilus*). Result showed high prevalence (57.14%), (54.16%) for the male *Hyalomma excavatum* and *H. anatolicum* viz 46%, 43% for the two female species respectively. While 100 percent prevalence were observed for male *Rhipicepalus* (*Boophilus*) *microplus* and *R. (B.) appendiculatus* respectively. Females of the two ticks species were not found in the present study. Moreover, total percent sex-wise count of ticks indicated 78.0% for male ticks compare to females (45.0%). It was concluded that population of tick parasites was high noted in the present study, and this could affect the prosperity and fecundity of chiltan wild goats. Therefore, to reduce ectoparasites burden and its impact on the fertility and health status, planning of strong policy aiming at creating awareness about the value of wildlife and control of ectoparasites with sustainable veterinary services is required.

SECTION - V

FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY AND MARINE BIOLOGY

1. ECOLOGY AND ENVIRONMENTAL POLLUTION

2. FRESHWATER BIOLOGY AND FISHERIES

3. MARINE BIOLOGY

4. PALAEOLOGY

5. WILDLIFE, DIVERSITY AND CONSERVATION

6. BIODIVERSITY

1. ECOLOGY AND ENVIRONMENTAL POLLUTION

ECOLOGICAL DISTRIBUTION OF SOIL MACRO-INVERTEBRATES IN DIFFERENT AGRO-ECOSYSTEMS

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Macro-invertebrates species are distributed randomly on earth. Their distribution varies according to the vegetation and abiotic factors pertaining in that specific ecological area. They play an important role in the balance of ecosystem. The present research work was conducted in district Faisalabad, Punjab, Pakistan to find out the ecological distribution of soil macro-invertebrates in different agro-ecosystems. Soil samples were collected from fish farm site and agro-field area. From the two ranches, overall, 1104 specimens were recorded. Highest population was recorded near fish farm cultivated area 60.96 % (N=673); While, lowest population was recorded from agriculture field 39.04% (N=431). Total 14 orders, 41 families, 54 genera and 68 species were observed from fish farm fields. Although, 12 orders, 34 families, 49 genera and 57 species were counted from the agro-fields. Highest abundance per sampling was recorded of 36 species at 15^o C temperature and 41% humidity. As compared to agro farm fields, highest population was recorded as 20 species at 30^oC temperature and 46% humidity. Highest species relative abundance from fish farm site was *Reduvius personatus* (Reduviidae) 5.65%, N=38. While, *Cylisticus convexus* (cylisticidae) was prevailing 6.03% (N=26) at agro-fields. Highest relative abundance of genus *Clubiona* 11.89% (N= 80) from fish farm fields. Relative abundance of family formicidae 17.40% (N=75) was highest at agro-farm fields. Maximum diversity H was (2.1683) from fish farm fields. Whereas, from agro-field was (2.1078). ANOVA results showed non-significant difference from fish farm and agro-fields (F=0.00; P=0.9505).

VERTICAL DIVERSITY AND ABUNDANCE OF SOIL MACROFAUNA IN DRY AND WET TERRITORY

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During present research “Vertical diversity and abundance of soil macro-fauna in dry and wet territory” was recorded at Faisalabad District. From (1-6' layer), maximum population was recorded 62.57% (N = 219) from Fish-farm; whereas, least population was recorded 37.43% (N = 131) from Agro-farm. However, in (7-12' layer), maximum population was recorded 69.29% (N = 185) from Fish-farm and least population was recorded 30.71% (N = 82) from Agro-farm; whereas in (13-18' layer), maximum population was recorded 80.09% (N = 177) from Fish-farm and least population was recorded 19.91% (N = 44) from Agro-farm. From 1-6 layer of fishfarm, relative abundance was recorded extraordinary for genus *Alaus* 15.53% (N = 34). From Agro-farm (1-6' layer), pest ratio was recorded up to 0.04% (N = 10) pertaining to following taxa: *Tribolium castaneum*, *Lyctus planicollis*, *Coptotrirmes formosae* and *Achroia grisella*. Whereas, from Fishfarm (1-6' layer), pest ratio was recorded up to 0.13% (N = 18). From Agro-farm (7-12' layer), pest population was recorded up to 0.05% (N =10) pertaining to following taxa: *Tribolium castaneum*, *Tenebrio molitor*, *Gonocephalum pusillum*, *Pentodon algerinus* and *Achroia grisella*. In contrast to Fish-farm (7-12' layer), pest population was recorded up to 0.13% (N=11). From Agro-farm (13-18' layer), pest ratio was recorded up to 0.01% (N=3). While, from Fish-farm (13-18' layer), pest ratio was recorded up to 0.11% (N =5). From Agro-farm (1-6' layer), predator ratio was recorded up to 10.505% (N = 23) and from Fish-

farm (1-6' layer), 25.19% (N = 33). From Agro-farm (7-12' layer), predator population was recorded up to 7.57% (N = 14) and from Fish-farm 30.48% (N = 25). From Agro-farm (13-18' layer), predator ratio was recorded up to 6.77% (N = 12) and from Fish-farm 25.00% (N = 11).

HUMAN CARNIVORE INTERACTIONS IN THE TERICHMIR FOOTHILLS OF CHITRAL PAKISTAN

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Chitral lies at the westernmost border of snow leopard range in Pakistan and contains several other important carnivore species, but fewer areas have been explored with underlying issues such as livestock predation, which negatively impacts conservation efforts. The study aims to present the occurrence of carnivore species in the Terichmir foothills of Chitral, Pakistan. The study also assesses livestock lost to predation and quantifies the consequent economic loss incurred by the community. Camera traps (Reconyx TM Hyperfire HC 500 and PC 900) were used to document the occurrence of carnivore species in the specified area. Semi-structured questionnaires were used to assess interactions between humans and carnivores. Five different species of carnivores including the snow leopard, jackal, fox, stone marten and wild cat were documented using camera traps. A questionnaire survey also revealed presence of wolf, lynx and leopard cat in the area. A total of 141 heads of livestock were killed by different carnivores, with an average of (0.87 animals per respondent) during 2010–2015. Jackals were responsible for the highest number of livestock losses (46% of the total), followed by wolves (32%). Together, these predations resulted in a loss of US\$ 10,710 to the communities. A further loss of US\$ 49,200 was incurred by the communities due to disease caused mortalities of 460 livestock heads. Views of most respondents were found positive towards carnivores. Interaction between different carnivore species and people dwelling in the Terichmir valley is occasional. Whereas carnivores incur an economic loss to the people by preying on their livestock, the key factor resulting in the major loss of livestock appears to be disease led mortalities.

ECOLOGICAL CO-EXISTENCE OF ORDER DIPTERA, COLEOPTERA, LEPIDOPTERA AND HYMENOPTERA AMONG DIFFERENT ECOSYSTEMS VIZ. AGRO-ECOSYSTEM, CITRUS ORCHARDS AND FISH FARM

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Diversity Indices are key components to draw the natural lines regarding taxa composition pertaining to any managed or unmanaged landscaping. The present research was depicted to compare “ecological co-existence of order Diptera, Coleoptera, Lepidoptera and Hymenoptera in various ecosystems viz. Citrus orchards, Agro-ecosystem and Fish farm” under the different ecological conditions of Faisalabad (Punjab), Pakistan. Overall, 3347 specimens were recorded, and maximum population was recorded from Agro-ecosystem 40.78% (N = 1365) followed by Fish Farm 30.92% (N = 1035) and Citrus Orchard 28.29% (N = 947). Maximum diversity (H') was observed from Agro-ecosystem (2.3413) related to Fish farm and Citrus orchard (2.2588), (2.2368) respectively. Value of evenness was extraordinary from Agro-ecosystem (0.1088) as compare to Fish farm (0.0858) and Citrus orchard (0.0795). Whereas Maximum dominance was recorded from Agro-ecosystem (1.1088) and lowest from Fish farm (1.0858) and Citrus orchard (1.0795). Richness having highest value in Citrus orchard (24.6671) as compared to Fish farm (22.6197) and Agro-ecosystem (21.1362). Analysis of Variance (ANOVA) among three fields (Agro-ecosystem, Citrus orchard and Fish farm) displayed non-significance outcomes (F=0.23; P=0.7977). t-test conscripted as a whole that foliage fauna of insects was occurred non-significantly in these three fields. Agroecosystem-Citrus (t-value = 1.44; P-value =

0.2449), Agroecosystem-Fish farm (t-value = 2.55; P-value = 0.0840) and Fish farm-Citrus (t-value = 0.46; P-value = 0.6783). Linear regression established that structural community and taxa composition were varied significantly among Agroecosystem-Citrus ($F = 42.20$; $P \leq 0.0229$), Agroecosystem-Fish farm ($F=220.59$; $P \leq 0.0045$) while, Citrus-Agroecosystem ($F = 20.05$; $P \leq 0.0464$).

THE CROSS-SECTIONAL STUDY ON THE PREVALENCE OF MALARIAL PARASITE IN A TERTIARY CARE HOSPITALS OF SINDH AND CASE STUDY OF MALARIA TREATMENT WITHOUT MEDICINE

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This cross sectional study was conducted by the different physicians and malaria case managers in the different tertiary care hospitals of Sindh in which the patients of malaria were included as malaria is a global health emergency prevalent in the third world countries especially in the tropical regions. It is well known that about 3-5 million deaths are reported annually due to the Malarial parasite globally. Pakistan is one of the 109 countries where Malaria is endemic. Malaria is caused by the species of *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium ovale*. In Pakistan *P.falciparum* and *P. vivax* are the prevalent infecting parasites. Pakistan the two major vector species are *Anopheles culicifacies* and *Anopheles stephensi*. *Anopheles* typically breeds in the natural water bodies with clean, slow moving, warm water, with sufficient aquatic vegetation. However, ecological requirements of particular species may deviate from these typical conditions; *An. stephensi* can easily breed in closed artificial containers and *An. claviger* prefer relatively cold water. There are different medicines used for the treatment of Malaria in Sindh. Artesunate, Chloroquine and other anti-malaria medicine are successful in its treatment but there is resistance to the said drugs is widespread, which needs a great solution for its long term eradication and treatment. In this cross sectional study, 200 patients of all age groups and genders were selected randomly with Malarial Parasite Positive cases. After diagnosis the patients were counseled to start this type of treatment for the best possible result with consent in written. There was not a single tablet of Chloroquine etc given to any of the patient under study. A new and unique treatment was experimentally given which was the mixture of tea leaves and the lime juice. The mixture was given to the patients to drink three times daily followed by the proper feeding of the meals. The patients were strictly observed, outreached and monitored to drink the prescribed material consecutively for 13 months. A good clinical outcome was obtained and all the patients got completely cured from Malaria. This study has opened the doors for the further research on the same topic.

2. FRESHWATER BIOLOGY AND FISHERIES

USE OF BIOTIC INDICES AS A TOOL TO ASSESS ECOLOGICAL STATUS OF HARNO STREAM, ABBOTTABAD

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The present study was conducted to evaluate the ecological status of Harno Stream, Abbottabad, using biotic indices applied on aquatic fauna. The sites A and B were studied from January to June 2017. Seven bioindicator orders of macrobenthos were selected including Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Odonata, Diptera and Mollusca. 11 biotic indices were used i.e Family Biotic (FBI) index, ratio of EPT to Chironomidae (EPT/C), Ephemeroptera, Plecoptera and Trichoptera (EPT) index, Ephemeroptera, Trichoptera and Odonata (ETO) index, %Diptera, Percent contribution of the Dominant Family (DF %), Simpson's diversity index, Stream Invertebrate Grade Number Average Level (SIGNAL), Community Loss Index (CLI), Biological Monitoring Working Party (BMWP) index and Average Score Per Taxon (ASPT). The overall result of indices regarded site A having excellent aquatic conditions while site B was found to be highly polluted. Functional feeding groups were identified and seven functional feeding group indices were worked out to determine ecological status of the river. The scrapers showed much abundance (55.05%) at site A while gatherer-collectors were dominant (72.8%) at site B downstream. The dominance of certain groups, low richness of predators and absence of shredders revealed ecological disturbances at both sites.

PARTIALLY REPLACEMENT OF FISH MEAL WITH BONE MEAL IS MORE ECONOMICAL FEED FOR *OREOCHROMIS NILOTICUS*

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A study was characterized to find the effect of 30% and 60% bone meal replacing the fish meal in feed on growth performance and body composition of *Oreochromis niloticu*. After two months, growth performance in the term of body weight and length, as well as whole body composition like fat, crude ash, carbohydrates were determined. Data was analysed by ANOVA and Tukey's test. Highly significant growth was observed ($p < 0.05$) as the final weight was 22.00 ± 0.87 , 17.00 ± 0.12 and 19.00 ± 0.12 (g) in T₀, T₁ and T₂ group respectively. The maximum weight gain was observed in experimental group T₃ followed by T₂ and T₁. Total gain in body length was measured as 4.5, 2.83 and 3.14 (cm) in T₀, T₁ and T₂ respectively. The greater FCR values were at 60% bone meal (4.41 ± 0.58) while control group showed lower FCR values (2.74 ± 0.25). It was observed that higher SGR was calculated in control group (2.30 ± 0.28) and lower in 30% bone meal (1.13 ± 0.07). The whole body composition of fish was also showed significant differences among treatments. Moisture contents decreased as bone meal was increased. The higher values of moisture content were noted as $57.11 \pm 0.01\%$ and lower moisture contents were at 60% bone meal ($56.58 \pm 0.01\%$). There were no significant differences in the crude protein at 30% and 60% bone meal. Total fat was higher in fish feed 30% $4.05 \pm 0.03\%$. While Crude ash and carbohydrates were higher in fish fed with 60% bone meal

and was lower in control group. It was concluded that bone meal can be used to partially replace fish meal in fish feed as bone meal has given better body composition as well as growth results. Our study suggested that partially replaced fish meal with bone meal is more economical for commercial fish hatcheries.

MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF THE MEDITERRANEAN THREE SPINED STICKLEBACKS LIVING IN THE CAMARGUE WETLAND (RHONE RIVER DELTA)

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Population heterogeneity and salinity acclimation capacities of southern threespined sticklebacks (*Gasterosteus aculeatus* L.) living in different saline habitats of the Camargue area (Rhône delta, northern Mediterranean coast) were investigated. Individuals from lagoons with different salinity ranges and from freshwater canals were exposed to seawater (SW; 30 ‰), brackish water (BW; 15 ‰), or freshwater (FW; 5 ‰). Morphological measurements of sub-adult fish sampled from 1993 to 2017 were determined from fish inhabiting in these different habitats. Also, oxygen consumption rates, gene expression of the $\alpha 1$ subunit ($\alpha 1a$ and $\alpha 1b$ NKA isoforms) and branchial $Na^+ / K^+ -ATPase$, NKA activity as an indicator of the fish osmoregulatory capacity were measured from these fish and after exposure to abrupt SW or FW transfers. At all the studied locations, only the low-plated *leirus* morphotype was observed with also limited morphological variations. No short-term effect of salinity could be detected on oxygen consumption from FW, BW, and SW fish. In these animals, gill NKA activity was salinity-dependent with also less NKA $\alpha 1b$ in FW- than in SW-fish. Ionocytes in FW-fish gills were located along the lamellae and at their base, whereas these cells were restricted to gill filaments in SW-fish. Finally, electron microscopy revealed three different types of apical structures for these ionocytes: a honeycomb-like structure and a dome shape in FW, and deeply encrypted in SW. Therefore, sticklebacks of the Camargue area living in contrasted saline conditions belong to a very homogenous euryhaline population and are not exposed to strong metabolic demands due to salinity changes.

STUDY OF SELECTED WATER QUALITY PARAMETERS OF RIVER INDUS AT GHAZI, HUND & KUND DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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The present study was conducted to evaluate selected water quality parameters of Indus River at three sites that are Ghazi, Hund and Kund, District Swabi, KPK Pakistan. The purpose of the study was to identify that weather such water was suitable for fish survival, growth and for reproduction or not. The present study was conducted for three months that are January, March and June (2016). In each month and from each site three samples were collected and was analyzed in the laboratory then the mean was calculated and concentration of each parameters were pH (7.95), Electrical conductivity (295 μ S/cm), Total Dissolved Solids (180ppm), Total Suspended Solids (30.77ppm), Total Hardness (134.02mg/l), Calcium Hardness (94.51mg/l), Magnesium Hardness (45.05 mg/l), Total Alkalinity (140.35 mg/l), P-Alkalinity (45.33 mg/l), Chloride (37.29 mg/l), Potassium (6.74 mg/l), Sodium (19.75 mg/l) and Sulphate (45.10 mg/l). Therefore, it was concluded that the concentration all the studied parameters at each site was in normal range except Total Suspended Solids and having no adverse effect on fish survival, growth and reproduction.

ENVIRONMENTAL IMPACT OF WATER QUALITY ON FISH PRODUCTION IN HALEJI LAKE, DISTRICT THATTA, SINDH, PAKISTAN

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The present studies to investigate the environmental impact of water quality on fish production in Haleji Lake, District Thatta, Sindh, Pakistan. During November 2017 to January 2018 the physicochemical parameter like temperature, pH, dissolved oxygen, Chloride, conductivity, salinity, and total dissolved solids were recorded fortnightly at 8:0AM in each sampling date through -out the study period. In the present study the temperature values were ranged between (20- 23C with mean 22 ± 1.0), pH (7.3-7.9 with mean 7.62 ± 0.09), DO (4.0 – 5.3 with mean 5.3 ± 0.19), Chloride (0.311- 0.375 with mean 0.346 ± 0.028) conductivity (950-1240 with mean 1162.5 ± 60.15), salinity (0.3) and total dissolved solid (410-549 with mean 525.7 ± 19.13) from all the sampling sites. In current investigations on the relationship of length and weight of 6 different species were presented, total 140 fish were caught between September 2017 and March 2018 from Haleji Lake District Thatta, Sindh. *Notopterus chitala*, *Mastacembelus armatus*, *Notopterus notopterus*, *Channa punctatus*, *Aorichthys aor* and *Oreochromis mossambicus* was enumerated at different length groups. The length weight relationships and correlation of co efficient was analyzed. In the present studies values of b varied from 3.74 *Notopterus chitala*, 2.97 (*Mastacembelus armatus* 2.12 *Notopterus notopterus* 2.24 *Channa punctatus* 2.48 *Aorichthys aor* 3.89 *Oreochromis mossambicus*). The studies reveled that *Oreochromis mossambicus* showed better growth and pursued cube law ($b=3.89$) followed by *Notopterus chitala* ($b=3.74$) while *Mastacembelus armatus* ($b=2.97$), *Aorichthys aor* ($b=2.48$) and *Channa punctatus* ($b=2.24$) exhibited closed to ideal. Length weight values and coefficient of condition showed ideal growth of six different species from Haleji Lake, District Thatta, Sindh, Pakistan. It is concluded that the different parameters used in the study revealed that the physico- chemical parameters in the Haleji Lake is considered to be safe limits (WHO 2012) and good to support the survival and production of aquatic environment especially fish.

SEASONAL VARIATION IN THE BIO - CHEMICAL COMPOSITION OF CATFISH *RITA RITA* FROM INDUS RIVER NEAR JAMSHORO SINDH PAKISTAN

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The present studies on the seasonal variation in the biochemical composition of catfish, *Rita rita* from River Indus near Jamshoro, Sindh, Pakistan was initiated from January 2016 to December 2017 during the summer and winter seasons. The biochemical analysis was determined in terms of moisture, protein, lipid, carbohydrate and ash from small, medium and large group for male and female. The results revealed that the mean moisture content of male was 85.28 ± 2.33 , 84.0 ± 2.17 and 84.37 ± 0.95 in winter while summer was recorded as 84.87 ± 1.41 , 83.47 ± 1.24 and $80.63\pm 0.33\%$ respectively. In case of female the moisture content 84.06 ± 3.06 and 82.73 ± 1.79 during winter season 85.55 ± 1.41 , 83.5 ± 1.08 and $80.83\pm0.23\%$ during summer season. The average values of protein were 4.12 ± 1.20 , 3.60 ± 0.65 and $3.33\pm 0.23\%$ in winter higher values 11.01 ± 1.76 , 10.59 ± 1.22 and $9.28\pm 1.47\%$ in summer months in male. In case female 3.38 ± 1.06 and 0.93 ± 0.46 in winter season 10.45 ± 1.73 , 10.53 ± 1.18 and $9.13\pm1.73\%$ during summer season the average of lipid content 0.56 ± 0.18 , 0.53 ± 0.05 and $0.61\pm0.07\%$ during winter season while in summer 3.72 ± 1.23 , 5.47 ± 1.63 and $6.9\pm1.16\%$ in male. While in female during winter the values were 1.68 ± 1.82 and $0.83\pm0.38\%$ and in summer 3.81 ± 1.22 , 5.65 ± 1.67 and $6.89\pm0.94\%$ respectively. For carbohydrate content in both season there were no -significant variation was noticed in male and female. The ash content in winter and summer season in male 9.76 ± 3.14 , 11.39 ± 2.8 and $12.76\pm2.04\%$ and 2.28 ± 0.53 , 2.5 ± 0.29 and $3.03\pm0.55\%$ respectively while the female in winter season 9.69 ± 2.41 and $14.77\pm2.01\%$ in summer months 2.4 ± 0.58 , 2.81 ± 0.52 and $3.19\pm0.63\%$. Finally it was concluded that there were non- significant variation in different bio- chemical parameters was observed

during winter at different length groups while in summer season male was found to be with higher protein values in different length group then that of the female.

REPRODUCTIVE PERIODICITY AND CONDITION FACTOR ANALYSES OF CATFISH, *RITA RITA* FROM RIVER INDUS NEAR JAMSHORO, SINDH, PAKISTAN

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The reproductive periodicity of catfish, *Rita rita* was elucidated terms of eggs size, determination of gonadosomatic index and fecundity from March 2014 to February 2015 from River Indus, Jamshoro. The size of egg in present study was found between 0.25 to 1.40 mm, it starts increasing during May – July with highest in July. The percentage of gonadosomatic index (GSI) was calculated and found varied between 0.10 to 2.80 and 0.25 to 12.15 in male and female respectively. The values of both eggs size and GSI found to be increasing during May to July and also showed one peak in July. The estimation of fecundity in the present study was based on 10 mature females ranged 33.0 to 41.0 cm and 450 g to 800 g in total length and weight respectively. The number of eggs in the present studies varied between 36350 to 90000 eggs. The maximum fecundity was from a fish with a total length 41.0 cm and 800 gram and minimum from a fish with total length of 33.0 cm and 450 gram. The number of egg present in per gram of body weight was 15.85 eggs and the number of egg per gram of ovary weight was 475.80 eggs. Fecundity was plotted with body parameters and it showed ideal relationship with gonad weight in comparison to body length and body weight. It was concluded from the above findings that the catfish, *Rita rita* (Hamilton) breed once in a year during the month of July. A full grown specimen about 1 kg weight could produce about 0.1 million eggs during the breeding season. It was perceived from the length weight analysis that the slope (b) values as observed from the equation ($b = 3.04, 3.63$ and 2.98) in case of male, female and combined sexes appeared to be alike and termed as ideal 3 (good). The b value showed ideal growth between the sexes of *Rita rita* from Indus River near Jamshoro. The maximum relative condition factor value was observed in female (1.10). On average, the female was found to be slightly better in condition (mean $K_n = 1.10$) than that of male (mean $K_n = 1.09$).

APPARENT DIGESTIBILITY COEFFICIENTS OF AGRI-WASTES AS FEED INGREDIENTS FED TO SILVER CARP

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Apparent digestibility coefficients (ADCs) for crude protein, crude fat and carbohydrates of three agri-wastes (pea peels, potato peels and wheat straw) were determined for freshwater fish silver carp. The feed ingredients were analyzed for proximate composition prior to the formulation of reference and test diets. One reference and three test diets were prepared containing 35 % protein by linear formulation method. The fish specimens were fed at the rate of 2 % body weight twice a day on reference (control) diet and test diets composed of 700 g/kg reference diet and 300 g/kg test ingredient. Chromic oxide (Cr_2O_3) was used as an inert indicator. After two hours of the feeding session, feces were collected using suction and pressing method. Water quality parameters (temperature, pH and dissolved

oxygen) were monitored on daily basis through digital meters. The collected fecal material from each aquarium were dried and stored for chemical analysis. After the termination of experiments, each fish specimen of each aquarium were weighed and then dissected. Different portion of intestine were taken and stored for digestive enzyme analysis. The apparent digestibility of protein for pea peel ($83.84 \pm 1.00\%$) was higher than potato peels ($65.6 \pm 0.43\%$) and wheat straw ($65.99 \pm 0.27\%$). The apparent lipid digestibility and apparent carbohydrates digestibility were high for pea peels up to $74.36 \pm 1.13\%$ and $86.63 \pm 0.27\%$, respectively. Final weight gain (%), specific growth rate (%) was higher for diet 1 with lower feed conversion ratio. Amylase and protease activity were higher at anterior portion and minimum at posterior portion of intestine. Knowledge of ADC values for these ingredients allow feed producers to develop nutritionally balanced, low-cost feed formulations for this species.

MORPHOMETRIC AND MERISTIC STUDY OF THE DIFFERENT PARAMETERS OF SCALES OF *CHANNA STRIATA* (BLOCH, 1793)

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The present research was carried out to study the morphometric and meristic characters of the scales of a freshwater fish, *Channa striata*. For this purpose, scales of the head region, lateral line, and caudal region were studied under the microscope. Results show that *C. striata* have cycloid scales. The head scales lacking the radii while lateral line scales and caudal scales have 7-20 and 9-18 radii respectively. The total length of the scale was observed as 4.0-8.3mm for head scales, 4.3-6.1mm for lateral line scales and 3.0-5.9mm for caudal scales. The width of scales of the head, lateral line, and the caudal region was examined as 5.0-9.9mm, 3.0-6.1mm and 1.7-4.0mm respectively. The oval or pear-shaped focus was present at the center of the scale. The distance of the focus from the posterior margin of the scale was ranging 2.9-6.0mm in head scales, 2.0-4.0mm in lateral line scales and 1.1-3.1mm in caudal scales.

TILAPIA FARMING IN BRACKISH WATER: ECONOMIC POTENTIALS

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The shortage of sweet water in many countries like Pakistan, together with the competition for it with agriculture and other urban activities has increased the pressure to develop aquaculture in brackish zones of Pakistan. Tilapia is an excellent candidate for fish culture in brackish zone due to their ability to tolerate wide range of salinity. Therefore, a field study was conducted at Aquaculture Research Pond, MNS-University of Agriculture Research Farm Jalalpur Pirwala to conclude the impacts of brackish water of Jalalpur Pirwala with EC 17 dsm^{-1} on adaptability of GIFT monosex Tilapia. GIFT monosex Tilapia fingerlings were obtained from Govt fish seed hatchery Mian Channu distt Khanewal. Experiment has shown that no mortality of GIFT monosex Tilapia fish seed was recorded. The fish seed stocked in pond with salty water have succeeded to grow at Jalalpur Pirwala farm. Environmental and climatic conditions of Jalalpur Pirwala are suitable for the growth of Tilapia. Total pond area dedicated for tilapia production in 2018 was 500m². Tilapia was cultured in last week of March and harvested in first week of December with total yield 320kg. Tilapia culture in saline waters is biologically sound. The choice of culture systems and selection of species for development of commercial production, ranging from extensive to super-intensive, depend on biological, socio-economic and environmental factors.

**MOLECULAR IDENTIFICATION OF TYPE SPECIMENS OF *SCHIZOTHORAX SKARDUENSIS*
PRESERVED AT STEPHENSON NATURAL HISTORY MUSEUM, DEPARTMENT OF
ZOOLOGY, GC UNIVERSITY, LAHORE**

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DNA barcoding is a useful technique which identifies the organisms based on the variation in the mitochondrial DNA gene, cytochrome c oxidase I (*MT-COI*). The type specimens of *Schizothorax skarduensis* were taken from Stephenson's Natural History Museum, GC University, Lahore. These specimens were collected from river Indus at Skardu, Pakistan in 1967, and the controversy about its being a separate species started. The present project was aimed at molecular level identification of *Schizothorax skarduensis*. For this purpose genomic DNA was extracted from gills, *MT-COI* gene was PCR amplified and resulting sequences were analyzed. The neighbour joining method was used to make phylogenetic tree for each species. The nodes in K2P distance-based NJ trees were supported by high bootstrap values (100%). DNA barcoding approach separated *Schizothorax skarduensis* from other schizothorax species by 0.42% distance. The mean pairwise K2P genetic distance of *Schizothorax skarduensis* was 0.067%. The average K2P distance estimation of intraspecific divergence in this study for *Schizothorax skarduensis* was 0.945%. It is concluded that the earlier described type specimens of *Schizothorax skarduensis* are different not only morphologically but are also new species on genetic basis.

**INVESTIGATION OF WATER QUALITY IN RIVER KUNHAR AT BALAKOT
KHYBER PAKHTUNKHWA, PAKISTAN**

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Unplanned urbanization and rapid growth of industrialization increase river pollution crisis in river ecosystem. The problem of water quality deterioration is mainly due to human activities such as discharge of industrial and sewage wastes and agricultural runoff which cause ecological damage and pose serious health hazards. According to four years water study conducted by the Pakistan Council of Research in Water Resources (PCRWR) during 2002-2006 concluded that 84-89% of the total sources of water in the country are contaminated. This study revealed that Punjab province contained 81, 91, 92, 90 and 90% unsafe water from 2002 to 2006 respectively. Balakot is situated in Hazara Division Khyber Pakhtunkhwa, Pakistan. This sampling station of the river Kunhar is polluted due to anthropogenic activities and natural processes. Water of this area is used by various purposes. This site is huge populated and river is contaminated by domesticated waste. Water samples were stored in clean and dry plastic bottles with screw caps and labeled. Some parameters were analyzed on the spots by using digital meters while others were examined by titration methods. During the field study the following water quality parameters were analyzed on the spot discussed in detail below. The dissolved oxygen of water samples was measured by digital DO meter (Model: EZDO-7031, Taiwan). The pH of water samples was measured by using pH meter (China). Electrical conductivity was measured by EC meter (China). Total Dissolved Solids was measured by Digital TDS meter (China). Temperature was measured by Digital Temperature meter (China). Some water quality parameters like Total Alkalinity, Calcium Hardness, Water Chlorides and Total Hardness were analyzed by titration methods. Parameters discussed in detail below. Water sample (25 ml) was titrated with a pre-standardized H₂SO₄ solution. Methyl orange was used as an indicator. The used acid solution was noted from the burette. The values were calculated by applying the following formula: Total Alkalinity = (Vml of H₂SO₄ + 25) 5000 x N. Where, Vml stands for the volume of acid solution which was used and measured in ml. N stands for normality of the acid solution. The above mentioned formula will give result for alkalinity in mg CaCO₃/L directly. 25ml sample was treated with 0.02N H₂SO₄

solution (corresponding to the alkalinity of the sample) followed by the adding of 3-4 drops of the $K_2Cr_2O_7$ solution (indicator). After that the solution was titrated with standard $AgNO_3$ solution (0.014 N) taken in particular sort of burette. The volume of silver nitrate solution was noted. The formula which was used as follow: Weight of Cl^- mg/L = (Atomic weight of $Cl^- \times VL \times N \times 10^2$) + 25 Where, VL indicates the volume of $AgNO_3$ solution used considered in liters and N stands for familiarity of $AgNO_3$ solution.

Clean titration flask was used for such purpose in which 25ml sample was taken along with 2ml NH_3/NH_4Cl buffer solution of pH=10 was also added. After shaking a small amount of solid Eriochrome black T, just enough for color change (as an indicator), was added with spatula. This started titrating against standard EDTA solution taken in burette after shaking. The used volume was noted as $CaCO_3$ mg/l. The formula which was used as follow: Molecular weight of $CaCO_3$ in 25ml sample = molecular weight of $CaCO_3 \times VL$ of EDTA $\times M$ of EDTA. Where VL indicates volume of EDTA solution used which was measured in liters and M indicates the molarities of EDTA solution. After this the calculation were prepared for the weight of $CaCO_3$ /l of sample as:

$$\text{Total hardness/l as} = \frac{VL \times M \times 100 \times 100}{\text{ml of sample}}$$

A study was conducted to explore the amount of water quality in River Kunhar at Balakot sampling station Khyber Pakhtunkhwa, Pakistan. In the current study water quality was analyzed on monthly bases for five years i.e., 2013 to 2018. The results recorded in the present study demonstrated that water quality parameters falls within the recommended permissible ranges. All the parameters were found suitable for aquatic life. Due to high temperature and low DO level this area was not suitable for Rainbow and brown trout's survival although recommended for the *Schizothorax* species. By anthropogenic and natural activities this area was mostly contaminated. The present investigation summarized that water of this site was suitable and recommended for the various purposes. All the parameters were recorded within the recommended permissible range. Water quality was contaminated by anthropogenic processes.

ICHTHYOFAUNAL DIVERSITY IN THE STREAMS OF DISTRICT SWAT, PAKISTAN

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Study was conducted to investigate the diversity, abundance, and distribution of ichthyofauna of different streams of Swat, Khyber Pakhtunkhwa, Pakistan. Total of 928 fish specimens were recorded from streams (Aronai, Nagoha and Hazara stream) of District Swat. During the study period 21 fish species were collected, belonging to 2 orders and 3 families. The order Cypriniformes was the dominant order represented by 95.23% (906 Specimens) followed by Mastacembeliformes with 4.76% (22 Specimens) of the total recorded fish specimens. The family Cyprinidae was represented by 6 genera and 10 species, Nemacheilidae comprising of 10 species, only 1 specie identified and Mastacembelidae was represented by 1 specie. A total of 21 fish species 12 species were identified i.e. *Crossocheilus latius*, *Crossocheilus diplocheilus*, *Barilius pakistanicus*, *Barilius vagra*, *Barilius modestus*, *Cyprinus carpio*, *Puntius waageni*, *Puntius chola*, *Tor putitora*, *Shizothorax plagiostomus*, *Mastacembilus armatus*, and *Triplophysa stoliczkae*. Most fish species recorded from these streams are economically important, but some are few in number which needs human influence for their conservation and management.

ISOLATION AND IDENTIFICATION OF BACTERIAL PATHOGENS FROM CULTURED AND RIVERINE *CYPRINUS CARPIO*

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Fish production is primarily affected by bacterial pathogens. An extensive diversity of viral, bacterial and parasitic diseases are assimilated with cultural fishes. Morbidities and mortalities in fish and fish products are perceived due to greater number of pathogenic bacterial species. The present research work was done to observe the infections in fish due to pathogenic bacterial species. For this purpose, infected samples of *Cyprinus carpio* were collected from Trimmu Head Works and Faisalabad Fish Seed Hatchery. Physico-chemical parameters of water were determined from both sampling sites. After Dissection skin, liver, gills and intestine was extracted and homogenized in Phosphate buffer. By the use of Streak plate and Pour plate method bacterial isolation was done after injecting the samples on nutrient agar plates. Pure bacterial culture was obtained by re-streaking of prominent colonies of bacteria. By applying physiological, biochemical and morphological characteristics bacteria was identified. Bacteria was classified as gram negative or gram positive through gram staining techniques. The quantitative study of bacterial vegetation resulting maximum bacterial load in gills (9.7×10^5 CFU/mL) of samples collected from Trimmu Head works and lowest bacterial count was observed in skin (0.69×10^4 CFU/mL) of samples collected from Faisalabad Fish Seed Hatchery. Gram staining, Catalase, Starch hydrolysis, Indole, Urease, Citrate utilization, Methyl red test, Fermentation and Voges paraskaur tests were performed to characterize the *Citrobacter diversus*, *Staphylococcus sp.* and *Bacillus cerus*. These pathogenic bacteria contaminate the fishes and causing bleedings, necrosis, lesions, swelling, and fatigue in experimental samples.

EFFECT OF DIFFERENT SUPPLEMENTARY FEED ON THE GROWTH OF COMMON CARP (*CYPRINUS CARPIO*)

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Growth performance of Common Carp were studied under four different feed supplements i.e. Feed contained Rice and Wheat bran (CG), Fish meal (T1), Poultry meal (T2) and waste proteins (T3). These four different supplementary feed were supplied to fish which were kept in four different tanks at Aquarium (Fisheries Lab) in Zoology department UAJ&K Muzaffarabad, at the rate of 0.25 gm/per day. Initially, at the time of stocking, the morph metric characteristics, viz, average body weight; average body length were measured. After initiation of experiment morph metric characteristics as physico-chemical factors of the water were recorded on daily basis throughout the study period. Average Body weight of Common Carp was recorded as, 13.91 ± 1.592 , 10.88 ± 0.617 , 11.96 ± 0.967 , 10.841 ± 0.725 gm under treatment T1, T2 and T3 and Control group respectively. The final body weight of fish in Control group and treatment groups showed that fish kept in T1 (P-Value=0.003) have gained the maximum body weight, While other treatment groups showed no significant statistical difference. Final Body Length of Common Carp were recorded as 7.44 ± 0.169 , 11.28 ± 1.407 , 8.031 ± 0.473 , 9.293 ± 1.490 cm under Control group, T1, T2 and T3 respectively. The final body length of fish in Control group and treatment groups showed that fish kept in T1 (P-Value = 0.001) gained more length followed by T3 (P-Value = 0.007). There is no significant statistical difference in Control group and T2.

IMPACT OF VARYING DIETARY PROTEIN COMPOSITIONS (%) ON GROWTH PERFORMANCE OF *LISSEMYS PUNCTATA*

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From the last several years, turtle population is declining rapidly due to destruction of habitat and breeding grounds, random hunting and illegal catching. The turtle species are being consumed as food in most parts of the world. Hence, their export could improve the economic status of the country if cultured at mass scale. Therefore, the current project was designed to evaluate the effect of varying dietary protein composition (%) on the growth performance of Indian flapshell turtle (*Lissemys punctata*). All the turtles were collected from the wild, transported and then transferred to the circular fiber glass tanks. Three groups of turtles (n=5) were fed for 90 days with artificial feed having 42% (control), 37% (T₁) and 31% (T₂) protein at ambient temperature (35°C), dissolved oxygen (5.5mgL⁻¹) and pH (7.5). The growth of Indian flapshell turtle was determined in terms of mean feed intake, weight gain, feed conversion ratio (FCR) and specific growth rate (SGR), on fortnightly basis. The data collected on all the growth parameters were subjected to statistical analyses by employing Statistical Analysis System (SAS^{9.1}). The results showed that statistically significant and highest mean feed intake was observed in the control group as compared to T₁ and T₂ groups. During growth trials, when the turtles were fed with 1% of their body weight, significantly (p<0.05) higher growth of turtles was recorded in T₂ group as compared to T₁ and control groups, in terms of weight gain, FCR and SGR.

ANNUAL RECORD OF FISH FAUNA OF CHASHMA LAKE DISTRICT MIANWALI, PUNJAB, PAKISTAN

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In current study a total of (1089) fish specimens of (21) species were collected from Chashma Lake on monthly basis. Fishing was prohibited in the June, July and August according to the Punjab fisheries department rules. The maximum no of catches were observed in December (165), January (153) and February (158). Twenty one species were belonging (5) orders, (8) families and (14) genera during one year study. Over all catches were dominated by *Labeo gonius*, *Cyprinus carpio*, *Wallago attu*, *Labeo rohita*, *Sperata sarwari*, *Channa marulius*, *Oreochromis mossambicus*, *Channa punctata*, *Cirrhinus reba*, *Labeo gonius*, and *Hypophthalmichthys molitrix* respectively with minimum. The Chashma reservoir is overall dominated by *Cyprinus carpio*, *Labeo gonius*, and *Wallago attu*.

WATER QUALITY PARAMETERS OF CHASHMA LAKE MIANWALI, PUNJAB, PAKISTAN

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One year study was carried out from April, 2016 to March, 2017 for the assessment of water quality parameters. It was observed from this study that range of Temperature(°C), pH, Total Dissolved Solids (mg/L) (TDS), Calcium (mg/L), Magnesium (mg/L) Total Hardness (mg/L), Total Alkalinity (mg/L), Chloride (mg/L), Conductivity (us/cm),

Nitrite (ppm), Nitrate-N (mg/L), Salinity (ppt), Bicarbonate (mg/L), Phosphate (mg/L) and Dissolved Oxygen (mg/L) of water of Chashma Lake was 8-31.5, 7.87-8.7, 80-96, 18-24, 18-26, 118-130, 85-142, 58-84, 144-154 0.12-1.2, 5.06-6.8, 0.2-0.7, 40-58, 0.11-0.9 and 7.5-8.8 respectively. Mean values of Temperature ($^{\circ}\text{C}$), pH, Total Dissolved Solids (mg/L) (TDS), Calcium (mg/L), Magnesium (mg/L) Total Hardness (mg/L), Total Alkalinity (mg/L), Chloride (mg/L), Conductivity (us/cm), Nitrite (ppm), Nitrate-N (mg/L), Salinity (ppt), Bicarbonate (mg/L), Phosphate (mg/L) and Dissolved Oxygen (mg/L) was also recorded that was 19.61, 8.27, 87.33, 21.66, 21.66, 123.8, 113, 73.58, 148.7, 0.583, 5.928, 0.476 49.8, 0.700 and 8.156 respectively. During this study maximum and minimum values of all above parameters were observed which was within the permissible range set by WHO.

BIOLOGICAL AND PHYSIOCHEMICAL ANALYSIS OF THE DRINKING WATER OF LOWER DIR

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Drinking water quality is a comparative word that relates water composition to the effects of anthropogenic activities and natural processes. Deterioration of drinking water quality is due to entering of chemical belongings into the H₂O resources through cross connection and leaks. Contamination includes organic materials, minerals and disease producing microbes. Heavy metals have combative effects on the health of humans and therefore their contamination of food chain justifies special attention. In current study the physio-chemical (Electrical conductivity, Temperature, pH and heavy metals) and bacteriological analysis of drinking water was conducted. A total of 28 water samples were collected from different water reservoir (e.g. wells, springs and hand pumps) of seven tehsils (Madan, Munda, Balambat, Timergara, Adenzai, Khall and Samarbagh) of district Dir (L). Samples were analysed for fecal coliform, total coliform and heavy metal detection. In 100 ml of water samples, the maximum number of total coliform (272, 190, 280, 230, 164, 224, 244), fecal coliform (120, 90, 120, 80, 85, 110, 95) and minimum number of total coliform (15, 36, 142, 130, 85, 175, 10), fecal coliform (00, 00, 45, 45, 38, 95, 00) were found collected from tehsils Madan, Munda, Balambat, Timergara, Adenzai, Khall and Samarbagh respectively. Heavy metal analysis showed higher concentration (mg/L) of selected heavy metals like copper (0.168), zinc (0.820), chromium (12), Lead (1.67) and Nickel (2.30). Concentration of metals were found in the drinking water samples of almost all the study sites of district Dir (L). It is concluded from the present research work that most of the H₂O samples of the study area were polluted with total coliforms, faecal coliform and higher concentration of heavy metals and are substandard as per World Health Organization standards for drinking H₂O.

FISH BIODIVERSITY AND PHYSIOCHEMICAL ASSESSMENT OF RIVER KURRAM AT DISTRICT BANNU KP, PAKISTAN

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Fish word derived from that animal which have skull, backbone and without limbs called non tetra pod craniates. Their gills are modified for respiration present throughout a life and limbs are replacing by the fins used for locomotion. The purpose of current study was to investigate the fish biodiversity and physiochemical assessment of river Kurram district Bannu. Water samples were taken in between 1.00 to 3.00 p.m. from two sites (Kurram Gari and Daud Shah) within a month of river Kurram and brought to the water monitoring laboratory National Agriculture Research Center (NARC) Islamabad for analyzed their hardness, alkalinity, pH (Power of Hydrogen ion

concentrations), TDS (total dissolved solids), temperature and oxygen. Total 8 species belonging 7 genera, 4 families and 4 orders were collected from two sites (Kurram Gari and Daud Shah) of river Kurram. The cyprinidae family was most abundant represented by 5 species, notopteridae, mastacemblida and channidae families were represented by 1 species. The catchment frequencies of the collected specimen were 49 and percentile value of cyprinidae (63.27%), mastacemblidae (14.28%), notopteridae (12.24%) and Channidae (10.21%) respectively, while most of the species were commercially important fishes. In river Kurram the highest values of alkalinity and hardness were in June and August with ranges of 186.5 ± 2.69 mg/L, 218.5 ± 4.5 mg/L respectively. The pH was recorded with values 8.26 ± 0.24 , April and 6.82 ± 0.1 , October. The maximum and minimum values of TDS were recorded in April and August 474.5 ± 3.5 ppm, and 324 ± 4.1 ppm respectively. The oxygen and temperature values (7.15 ± 0.14 mg/L, 27.8 ± 0.06 °C) were considered maximum in months of May and July, while the minimum values (4.3 ± 0.1 mg/L, 13.1 ± 0.05 °C) were recorded in months of June and January, the remaining months have moderate values. From the current study it may be concluded that the River Kurram have rich fish fauna. After analyzing their physiochemical parameters showed the normal values and not too much different and risky for fish life in the river Kurram. The department of the fisheries should brought new species in the water bodies to enhance the fish biodiversity because it is the evidence to provide proteins to the human population.

COMPARATIVE STUDY OF DIFFERENT BODY INDICES AND CONDITION FACTOR (K) OF WILD AND HATCHERY REARED RAINBOW (*ONCORHYNCHUS MYKISS*) AND BROWN TROUT (*SALMO TRUTTA FARIO*) OF SWAT, KP, PAKISTAN

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Study of different body indices and condition factor of fish represents a way of monitoring the environmental factors influence on fish body growth and health status. The present study was conducted for the comparison of different body indices i-e Viscera-somatic index (VSI), Hepato-somatic index (HSI) and Condition factor (K) of hatchery reared and wild rainbow and brown trout. Samples of both species were collected from River Swat Kalam and Madyan Trout Hatchery during month of September. Significant differences ($P < 0.05$) were observed in VSI and HSI values which shows direct relationship with increasing weight and size both. Values for VSI was significantly different ($P < 0.05$) among all the groups. Also, significantly ($P < 0.05$) higher VSI values were recorded for hatchery reared Rainbow and Brown trout as compared to wild. Similarly, the values of HSI were significantly different ($P < 0.05$). Significantly higher HSI values were observed in wild Rainbow and Brown trout. Among these significantly different ($P < 0.05$) HSI value were recorded for wild brown trout. Although, significant differences ($P < 0.05$) were greatly observed in the values of Condition factor of hatchery reared and wild Rainbow and Brown trout, respectively. In both species collected from hatchery, condition factor values were significantly higher ($P < 0.05$) while in case of wild collected samples brown trout have higher condition factor values than rainbow trout population.

CONTRIBUTION OF AQUACULTURE SECTOR IN ECONOMY OF PAKISTAN

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Aquaculture management and resources are very important in the maintenance of economy of a country. Any irregularity in the management of these fields may disturb the economy of a country. Economically, stable aquaculture management requires the revolution of public property through a controlled access system can raise profits. Present state of Pakistan's aquaculture trade shows the ecological background of our country and clarify

recent advances in technology and their influence on the capture and catch. The factors contributing in progress comprise administration efforts, fleet increase and expansion of trade markets. To progress in aquaculture, Pakistan should require balanced resource management. This study also emphasizes on problems to sustain suitable management strategy for the regulation of the Pakistan's aquaculture industry. Pakistan contribute about 0.25% which is the lowest in the world export market. Pakistan can get benefit from resources and expand this sector by implementing modern techniques because nature has provided sufficient resources that might be utilized to stimulate Pakistan's exports.

BREEDING BIOLOGY OF SNAKEHEAD *CHANNA MARULIUS* FROM INDUS RIVER JAMSHORO, SINDH, PAKISTAN

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Present investigation on breeding biology of Snakehead channa marulius was conducted in the month of March 2016 to August 2016, 300 specimen were observed, sized ranged from 43.8 to 71 cm in total length and 750 g to 3100 g in weight respectively, values of Gondsomatic Index was observed highest 0.52 and 1.18 in male and female respectively in the month of July. Monthly ova diameter measurement showed progressive increase from March to July (1.13 mm) peak in July. Fecundity was estimated highest 18095 from a fish of total length of 71 cm at the gonad weight of 77 and lowest 2070 eggs from the fish of total length 46.5 cm at gonad weight of 6.5 g and posses strong relationship with gonad weight.

HEALTH RISK ASSESSMENT AND ESTIMATION OF HEAVY METALS IN RIVER SIRAN AT ICHERRIAN KHYBER PAKHTUNKHWA, PAKISTAN

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The harmful effect of trace elements when consumed above the recommended limit can be toxic (acute, chronic or sub-chronic), and heavy metals can be neurotoxic, carcinogenic, mutagenic or teratogenic. The general symptoms of humans related to metal [e.g., Cd, Pb, As, Hg, Zn, Cu and aluminium (Al)] poisoning include vomiting, convulsions, paralysis, ataxia, hemoglobinuria, gastrointestinal disorder, diarrhoea, stomatitis, tremor, depression and pneumonia. Usually in unaffected environments, the concentration of most of the metals is very low and is mostly derived from the mineralogy and the weathering. These elements have attracted particular consideration in the recent two decades and within the framework of environmental investigation. Pollution of the natural environment by trace elements is a worldwide problem. These metals are indestructible because of their resistance to decomposition in natural condition. Icherrian is very imported spot of the River Siran located in Hazara Division Kyber Pakhtunkhwa Pakistan. This area is very suitable for other invertebrates and vertebrates fauna. Lake Shah Ziab is situated in it upper side which is one of the most attractive place. Water velocity is slow in this area. Water samples were stored in clean and dry plastic bottles with screw caps and labeled. The freshly collected samples were analyzed for Heavy metals analysis at GC University Faisalabad lab by using atomic absorption The stock solution was prepared as 1000 ppm = 1000 mg/l. Then 100 ppm solution was prepared from stock solution using serial dilution equation of $C_1V_1 = C_2V_2$ The water samples were first filtered with the help of filter paper and then taken in 250 ml of glass bottles and subjected to the atomic absorption spectrophotometer (Zn, Cu, Cd, Mn, Cr, Pb) at GC University Faisalabad lab. The present examination was intended to monitor the amount of heavy metals such as Pb, Cd, Zn, Cr, Cu and Mn in

River Siran at Icherrian sampling station Khyber Pakhtunkhwa, Pakistan. For this reason six heavy metals were chosen to examine heavy metals concentration. The recorded heavy metals from the current study contain Cu 1.06-1.31, Cd 0.09-1.31, Pd 0.04-1.28 and Cr 0.03-0.15. These metals were above the permissible range while Zn 1.17-1.78 and Mn 0.04-0.09 were lies within the permissible ranges. The main goal of the current analysis was to examine heavy metals concentration in River Siran at Icherrian Jalkhad site Khyber Pakhtunkhwa Pakistan. The study reviled that maximum heavy metals crossing the permissible limits which are not good for health.

QUANTITATIVE ANALYSIS OF HEAVY METALS IN RIVER SIRAN AT JABORI KHYBER PAKHTUNKHWA, PAKISTAN

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Increasing human influences through heavy metal pollution have over the years led to the depletion of our aquatic biodiversity. As a result, several important endemic fish species have become threatened. Realizing this, concern for assessment of trace or heavy metals in fish species in most of our water bodies have increasingly been gaining ground throughout the word. The characteristic feature of heavy metal is their strong attraction to biological tissues and in general their slow elimination from biological systems. They are environmentally stable, non-degradable and induce toxic effects. Environmental pollution is a worldwide problem, heavy metals belonging to the most important pollutants. The progress of industries has led to increased emission of pollutants into ecosystems. Jabori site of the river Siran is also important picnic spot. This zone of the sampling point is beautiful and attractive. Due to green fields of this site majority of tourists visit to this site. Due to anthropogenic activities water quality is badly affected. In this spot of the river variety of Ichthyo fauna existing. Water samples were stored in clean and dry plastic bottles with screw caps and labeled. The freshly collected samples were analyzed for Heavy metals analysis at GC University Faisalabad lab by using atomic absorption The stock solution was prepared as 1000 ppm = 1000 mg/l. Then 100 ppm solution was prepared from stock solution using serial dilution equation of $C_1V_1 = C_2V_2$ The water samples were first filtered with the help of filter paper and then taken in 250 ml of glass bottles and subjected to the atomic absorption spectrophotometer (Zn, Cu, Cd, Mn, Cr, Pb) at GC University Faisalabad lab. The present investigation was designed to analyze the amount of heavy metals in River Siran at Jabori sampling station Khyber Pakhtunkhwa, Pakistan. In the current study three sampling stations were selected i.e. (Upstream, Mid Point and Downstream) in River Siran at Jabori which were away from one another 100 meter distance. The main goal of the current research was to assess heavy metals such as Zn, Cu, Cd, Pb, Cr and Mn in the River Siran at jbori sampling station. The present research conducted in River Siran at Jabori site Khyber Pakhtunkhwa Pakistan revealed that this area water quality is contaminated via heavy metals which might be enter to water bodies by the tourism activities etc. Therefore, to avoid the heavy metals contamination Environmental Protection Agency have to controlled heavy load of tourism.

ESTIMATION OF WATER QUALITY IN RIVER KUNHAR AT RARA KHYBER PAKHTUNKHWA, PAKISTAN

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The term limnology is derived from the Greek word limne meaning pool, marsh, or lake. The science arise from lake investigation but now it includes both the study of lotic habitats (running water) and lentic habitats (standing

water). Contaminated sediments are another significant source of water pollution. These may be derived from inputs of suspended solids to which toxic substances are absorbed; such as soil particles in surface water run-off from fields treated with pesticides. Pesticides are useful tools in agriculture but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored. Water covers 71% of the earth surface and make up 65% human body. Nowadays, 400050 Million populations in 29 different countries is facing the problem of water shortages. River Kunhar confluence to river Jehlum at Rara station of the study. This area is very green and comprising variety of flora and fauna. The mountains of this area are rich of forest which provides a good habitat for wildlife. Water samples were stored in clean and dry plastic bottles with screw caps and labeled. Some parameters were analyzed on the spots by using digital meters while other were examined by titration methods. During the field study the following water quality parameters were analyzed on the spot discussed in detail below. The dissolved oxygen of water samples was measured by digital DO meter (Model: EZDO-7031. Taiwan). The pH of water samples was measured by using pH meter (China). Electrical conductivity was measured by EC meter (China). Total Dissolved Solids was measured by Digital TDS meter (China). Temperature was measured by Digital Temperature meter (China). Some water quality parameters like Total Alkalinity, Calcium Hardness, Water Chlorides and Total Hardness were analyzed by titration methods. Parameters discussed in detail below. Water sample (25 ml) was titrated with a pre-standardized H_2SO_4 solution. Methyl orange was used as an indicator. The used acid solution was noted from the burette. The values were calculated by applying the following formula: Total Alkalinity = (Vml of $\text{H}_2\text{SO}_4 + 25$) 5000 x N. Where, Vml stands for the volume of acid solution which was used and measured in ml. N stands for familiarity of the acid solution. The above mentioned formula will give result for alkalinity in mg CaCO_3/L directly. 25ml sample was treated with 0.02N H_2SO_4 solution (corresponding to the alkalinity of the sample) followed by the adding of 3-4 drops of the $\text{K}_2\text{Cr}_2\text{O}_7$ solution (indicator). After that the solution was titrated with standard AgNO_3 solution (0.014 N) taken in particular sort of burette. The volume of silver nitrate solution was noted. The formula which was used as follow: Weight of Cl^- mg/L = (Atomic weight of $\text{Cl}^- \times \text{VL} \times \text{N} \times 10^2$) + 25 Where, VL indicates the volume of AgNO_3 solution used considered in liters and N stands for familiarity of AgNO_3 solution. Clean titration flask was used for such purpose in which 25ml sample was taken along with 2ml $\text{NH}_3/\text{NH}_4\text{Cl}$ buffer solution of pH=10 was also added. After shaking a small amount of solid Eriochrome black T, just enough for color change (as an indicator), was added with spatula. This started titrating against standard EDTA solution taken in burette after shaking. The used volume was noted as CaCO_3 mg/l. The formula which was used as follow: Molecular weight of CaCO_3 in 25ml sample = molecular weight of $\text{CaCO}_3 \times \text{VL}$ of EDTA \times M of EDTA. Where VL indicates volume of EDTA solution used which was measured in liters and M indicates the molarities of EDTA solution. After this the calculation were prepared for the weight of CaCO_3/l of sample as:

$$\text{Total hardness/l as} = \frac{\text{VL} \times \text{M} \times 100 \times 100}{\text{Ml of sample}}$$

A research work was carried out to evaluate water quality of River Kunhar at Rara sampling station Khyber Pakhtunkhwa, Pakistan. Duration of the study was 5 years. For this purpose eight water quality parameters were chosen. Some parameters were analyzed on the spot by using digital meters while other by titration methods. The recorded results of the current study were in the range of Temperature ($^{\circ}\text{C}$) 14.46 ± 2.18 - 17.34 ± 2.61 ; pH 6.51 ± 0.34 - 7.66 ± 0.14 ; DO (mg/l) 12.37 ± 2.64 - 15.44 ± 1.35 ; TDS 212.46 ± 37.03 - 249.78 ± 57.04 ; Total Alkalinity (mg/l) 182.58 ± 31.02 - 235.86 ± 26.05 ; Total Hardness (mg/l) 50.45 ± 11.85 - 60.26 ± 14.38 and Chlorides (mg/l) 23.56 ± 5.43 - 52.82 ± 8.42 respectively. In the present study all the water quality parameters were found within the recommended ranges. From this research conducted in river Kunhar at Rara can be revealed that water quality was suitable for aquatic life. In the future, this site may be used for wildlife. Natural processes such as rocks erosion alter the water quality.

INVESTIGATION OF SOME BIOCHEMICAL ASPECTS IN EDIBLE TISSUES OF SOLE FISHES ALONG THE COAST OF PAKISTAN

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The Sole fishes are mostly common in various habitats of Pakistan coastal areas and belong to three families (Paralichthyidae, Cynoglossidae and Soleidae), which considered in this study. Most species are indigenous to the Indo-Pacific region, however they also occur in warmer parts of the East Atlantic. The shallow waters of a muddy or sandy bottom, including estuaries are the preferable habitat of these species, but a few species are restricted to fresh water. The current study is based on some biochemical study of sole fishes collected from five fish harbors (i.e., Karachi, Korangi, Ketu Bandar, Ormara, and Pasni) along the coast of Pakistan. The six species of sole fishes i.e., *Cynoglossus puncticeps*, *Cynoglossus arel*, *Pseudorhombus javanicus*, *Pseudorhombus elevatus*, *Cynoglossus quadrilineatus*, *Euryglossa orientalis* are abundantly occurring along the coast were collected from various fish harbors and analyzed for biochemical aspects (moisture, protein, lipid and carbohydrate levels). The biochemical analysis revealed that the high protein and lipid contents in *P. elevatus* collected from the Karachi fish harbor. The highest carbohydrates observed in *P. javanicus* from Ketu Bandar.

COMPARATIVE STUDY OF ECOTOXICITY OF CLEAN AND POLLUTED WATER OF TARBELA DAM KPK PAKISTAN

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Water is essential for supporting a productive environment for all living organisms. However, agricultural chemicals, industrial wastes, domestic sewage and municipal wastewater have considerably increased the contamination of water resources, which can affect the living organisms. Therefore, the present study was conducted for the assessment of ecotoxicity of clean and polluted water of Tarbela dam. The water samples were collected from six different sites three each from clean and polluted water sites of Tarbela dam. Water samples were analyzed through biotest ECOTOX in which *E. gracilis* used as a biotest organism. Different parameters of *E. gracilis* such as motility, swimming velocity, gravitactic orientation and cell shape were used as end points. Polluted water samples affected various parameters of *E. gracilis*. Motility was most sensitive parameter significantly inhibited by polluted water samples of Soha, Isharah Chowk and Kalabat followed by upward movement and Velocity that was also significantly inhibited by these polluted water samples. Cell compactness was also inhibited to some extent by polluted water samples however, r-value was not significantly inhibited by any of the water samples. From the inhibitory response of all the parameters it was seen that polluted water sample from Isharah Chowk was most toxic followed by Soha. The clean water sample of Chandi Chowk showed more positive effects by further enhancing the movement of *E. gracilis* followed by clean water samples of Chakai and Darwaza.

STUDY ON FOOD FEEDING OF INDIAN GLASS FISH PARAMBASSIS RANGA (HAMILTON 1822) FROM INDUS RIVER JAMSHORO, SINDH, PAKISTAN

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The samples of *Parambassis ranga* were collected on monthly basis from Indus River near Jamshoro during the

month of February-July 2017. A total of 129 specimens of *Parambassis ranga* (58 males and 71 female) having total length (TL) 2.5-7.8 cm (males) and 1.9-8.0 cm (females), weighing 0.7-5.2 g (males) while females 0.1-6.2 g. All fish samples were brought to laboratory of Department of Fresh Water Biology and Fisheries for further studies. study revealed that it is larvivores and carnivores fish. It has great preference for copepods. And also feeds on prawns' insects.

POPULATION DYNAMICS OF MONSOON RIVER PRAWN *MACROBRACHIUM MALCOLMSONII* ALONG THE REACHES OF LOWER INDUS RIVER

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Macrobrachium malcolmsonii commonly known as Monsoon River prawn, is commercially important species in Indian subcontinent. It is second largest freshwater prawn after *M. rosenbergii*. This is migratory species and its life cycle traits depend on riverine flows towards sea, due to estuarine/marine derived larval development. Scarcity of Freshwater in Indus River has adversely effected population of migratory species (fish / prawn) in Indus River. The study aims to clear the population status of *M. malcolmsonii* in Indus River. Sampling of *M. malcolmsonii* was conducted from Fish Market Thatta from April 2017 to September 2017 and from March 2018 to September 2018. Where catches were brought from the lower reaches of Indus River. Samples were brought to the laboratory of Freshwater Biology and Fisheries, University of Sindh for further examination, including total length (cm), total weight (g), fecundity. In total 498 specimen of *M. malcolmsonii* were used for the study, including 306 females and 192 males. Percentage of Female and male was 61.44 and 38.55, respectively indicating the clear dominance of female population over male. Length of female and male ranged between 7.9-23.4 cm and 5.8 to 25 cm, respectively. Ovigorous females dominantly appeared from April to September 2017, and March to September 2018. Fecundity was estimated from 132 females, using volumetric method. Fecundity ranged between 1570 to 16500. Our findings indicate that reproductive season of the species is from April to September. *Macrobrachium malcolmsonii* appears highly fecund species in Indus River, with female population dominating the male in number.

ARTIFICIAL BREEDING OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) USING VARYING DOSES OF OVAPRIM

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Induce breeding of African catfish (*Clarias gariepinus*) was carried out to study the effects of varying dose of synthetic Hormone Ovaprim. 24 Male and 24 Female fishes, total 48, were used during study. Weight of female fish was 2kg to 2.7 kg and that of male was from 1.98 to 2.7 kg. All broods were selected randomly and all groups consist of 4 male & 4 female per replica. A dose of 0.3, 0.4, 0.5, 0.6 and 0.7 ml/kg was injected to female fishes in five standard groups (B-F) respectively and male fishes with 0.2 ml/kg body weight while fishes of control group (A) were injected with normal saline water. Assessment was based on latency period, Egg mass, stripping (%), Fecundity, relative fecundity, fertilization (%), Incubation, hatching (%) and survival rate. Results obtained were clear that all these parameters, to assess the effects of different doses of Ovaprim, were highest with mean value of 10.18± 0.16, 280.50±0.86, 13.87 ±0.02, 196350 ± 606, 9710.25 ±19.93, 86.75± 1.25, 22.48 ±0.04, 86.13 ± 0.51 and 83.15± 0.43 respectively at the dose level of 0.4 ml/kg body weight among all tested doses but there was no significant difference statistically. It has been concluded that 0.4 ml/kg body weight Ovaprim was best for artificial breeding of *Clarias gariepinus* during study.

EFFECT OF DIETARY SUPPLEMENTATION BY PROBIOTICS ISOLATED FROM THE INTESTINE OF FISH ON SURVIVAL, GROWTH PERFORMANCE AND DISEASE RESISTANCE IN JUVENILE *Oreochromis niloticus*

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The intensification of aquaculture to solve the problem of hunger and protein malnutrition, international trade, the import and export of aquatic animals, climate change and environmental conditions are factors responsible for the emergence and the rapid spread of certain viral and bacterial infections in aquaculture that can cause significant economic losses. The improper and inappropriate use of antibiotics and other chemical compounds in aquaculture to prevent or control infections remains an indisputable problem of environmental pollution, antibiotic resistance in bacteria and diseases. The application of probiotics in aquaculture for some years remains a promising alternative to limit the development of this undesirable microflora, improvement of the health and the increase of the production of aquatic animals. However, several probiotics currently used are from other sources than the aquaculture sector. The aim of this study was to evaluate the effects of probiotic *Lactobacillus plantarum* 1KMT and *Lactococcus lactis* subsp. *lactis* 3FT, isolated from *Oreochromis niloticus*, on survival, growth performance, and disease resistance in the fish. The viability of probiotic strains was studied in the basal diet to determine the time required for feed reformulation. Ninety juveniles of monosex Nile tilapia, of average weight 10.99 ± 1.1 g, were divided into three groups in duplicate and put in aquariums of about 100 l: group G1 received the basal diet without probiotics (control), groups G2 and G3 were fed with basal diet supplemented with 1×10^8 cfu/g probiotics strains 3FT and 1KMT respectively. Each group received the food twice a day at 10am and 4pm. weight gain and microbiological analysis of fish droppings were evaluated every ten days during the experiment. After sixty days of experience, the survival rate and resistance of fish against infection with *Vibrio parahaemolyticus* were evaluated. The results showed that the bacterial load of our probiotic strains was maintained at 1.10^8 cfu/g for seven days in the basal diet. The survival rate was 100%, 96% and 86% in groups G3, G2 and G1 respectively. The final average weight in was 30.15 ± 5.95 g for G3, 26.3 ± 3.39 g for G2 and 23.7 ± 4.75 g for G1. Specific growth rates (SGR) and dietary conversion rates (DCR) were also higher in the G3, followed by G2 and G1. Survival rates after *Vibrio parahaemolyticus* infection for 14 days were 96%, 84% and 30% for G3, G2 and G1 respectively. This study showed that increased digestibility of nutrients by probiotics improves weight gain, survival rate, and fish resistance to infections.

COMPARISON OF DIETARY PROTEIN REQUIREMENTS AND NUTRIENT UTILIZATION OF *ACANTHOPAGRES BERDA* (FORSSKAL, 1775), UNDER DIFFERENT REARING CONDITIONS

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This study reports about the comparison of dietary protein requirements of, *Acanthopagrus berda* (weight 10.0-235 g) reared in different rearing conditions (tanks and ponds). From Sonari channel Hub River, Hawksbay, fish were collected and were reared in two separate trials. In experiment first, fish were kept in recirculating water tanks system while in experiment second fish were reared in earthen ponds. Four experimental feeds were formulated having 20%, 30%, 40% and 50% protein level, and were fed at feeding level of 2% body weight for 120 days in both the rearing condition. Higher percent weight gain and growth performance were noted in the fish fed with protein level of 40% and 50% in earthen pond while fish reared in tanks with same feed showed poor performance. The FCR of fish reared in ponds were better as compare to fish kept in tanks. The biochemical constituents of whole body revealed that, lipid and protein constituents of fish treated in pond with 40% and 50% was higher than fish kept in tanks with same feed. The hepatosomatic index (HSI) and Viscerosomatic index of fish treated with 40% and 50% protein level in earthen pond was also healthier than that of fish kept in tanks. For the quality assessment of fish meat and health aspect, the chemical composition of muscle, viscera and liver of the fish were also checked, which was not significantly affected by the culture conditions. These findings concluded that *A. berda* (weight 10.0 – 235g) showed best growth performance in ponds than in tanks with artificial feed having 40% to 50% protein level.

3. MARINE BIOLOGY

BIOECOLOGY OF PERIWINKLE *ECHINOLITTORINA OMANENSIS* (CLASS LITTORINIDAE) ON HIGH WATER MARK AT CLIFTON BEACH, KARACHI

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The *E.omanensis* were recorded first time in 2004 from Somalia gulf of Aden, north western Arabian sea of Pakistan by Reid, 2004. Littorinid gastropods are the important mollusc found at high tidal level on hard substrate of sea shore of Pakistan During the present studies it was observed that *E.omanensis* is the species found at intertidal zone at break water wall of Clifton beach Karachi, feed on algal bloom of marine water, the population of the species were less on wall as compare to the rocks. The distribution of *E.omanensis* vary according to the tidal level, when tide were high echinolittorina get sufficient food the population were increases, if the tidal level were low the population faces the problem of food scarcity due to this reason population of the echinolittorina were decreases. For abundance and distribution quadrature method were used. Total 2117 sample from June2018-Nov2018were collected. Length measured in cm and weight in mg. The minimum length of *E.omanensis* was 0.1 weight of this length vary 0.3 to 1.0 mg, and the maximum length was 1cm weight on this length vary between 100 to 130mg. Other testing also performed on such as correlation and anova. The results of correlation were strongly correlated in every month. In anova some months means of length and weight were same and in some months means do not same. This is the first ever record of *E.omanensis* in Karachi, Pakistan, to evaluate the distribution and abundance of the species at Clifton beach.

BLOOM OF *NOCTILUCA SCINTILLANS* IN GADANI (BALOCHISTAN COAST)

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The present study reports formation of bloom of the *Noctiluca scintillans* (dinoflagellate) in Gadani, Baluchistan coast. *Noctiluca scintillans* is a large, oblong, bioluminescent heterotrophic, non-toxic dinoflagellate. Blooms by *N. scintillans* are red or green associated with anoxia by feeding on toxigenic microalgae *N. scintillans* may transfer toxins to higher trophic levels and responsible for the mortality of fish and benthic fauna. Although blooms have been considered a consequence of upwelling in the summer season but we observed bloom in winter in Gadani. The increased frequency of algal blooms in the coastal areas is the result of human alterations to the coastal zone. Temperature, currents, wind and nutrients also play important part in the outbreak of an algal bloom. Algal blooms caused massive fish kill, affect fishery resources, biodiversity and vulnerable to the marine environment. As algal blooms are accompanied by severe impacts on coastal resources, economies and public health Government should develop environmental protection strategy for controlling pollution.

ANALYSIS OF MICROPLASTIC CONCENTRATION AT CLIFTON BEACH KARACHI

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Microplastic particles cannot see by naked eyes because of their size approximately about 0.005 mm. the concentration of plastic pollution is high at very famous tourist spot "Clifton beach, Karachi". Ten stations were

targeted in this study, from Bilawal House to Do Darya with equal distance of about 1 km and collected 1 gm sample from each station. By shaking and separation funnel method different forms, shapes and size are found. The data conducted in twelve months duration, from June 2017 to May 2018. Average graphs of station and month wise data were presented through this study. Microplastic chemical nature is very harmful for the environment and because of its hazardous effect many marine animals have been endangered. Marine fauna by ingesting microplastic facing many problems even whales and sharks are dying just because to take microplastic in their food. This study is very helpful to give knowledge about the presence and distribution of microplastic along the Clifton coast, Karachi which can be used for authorities, students and researchers for further issues and situation wise analysis.

FORAMINIFERA AS BIO-INDICATORS FOR MARINE POLLUTION IN GADANI SHIP BREAKING AREA

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In this study we have demonstrated the significance of foraminifera in detecting marine ecosystem pollution. Marine coastal areas of Pakistan are threatened by various anthropogenic activities from Industrial waste, domestic sewage, agricultural activities and dredging activities. The study of foraminifera is a valuable tool in modern geosciences. In the last decades foraminifera are very helpful in determining the environmental pollution research. The aim of ecological study is to prove the relationship between the biota and the marine environment. We observed these species of foraminifera, *Ammonia tepida*, *Elphidium sp.*, *Spiroloculina*, *Quinqueloculina lamarckiana*, *Cornuspira planorbis*, *Cornuspira involvens*, *Pseudononion sp.*, *Bolivina vadesens*, *Caucasina schichkinskye etc.*, in Gadani ship breaking area. The foraminifera are resistant to pollution can tolerate high levels of heavy metal and accumulated organic matter.

GREEN MUSSELS FISHERY IN CREEKS OF SINDH COASTAL AREA THATTA, PAKISTAN

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Green mussels are occurring in intertidal or shallow waters in attach form with various hard masses, mangroves trunks, stones, prop roots, heaps of stones, walls, rocks and sides of creeks. The green mussels are being collected during low tides when mudflat become exposed in the creeks Khai, Khudi, Pettiani, Ambra, Hajamro, Khobber in area of coastal taluka Ghorabari, Gharuho, Keti Bandar, Bhaghan, Kharo Chaan and Shah Bandar. In the collection of mussels all members of community are involved although they are not consuming as fodder but collecting as income and livelihood source. Size about 4 to 9 cm and weight 40 to 100 gram. It was observed that one family can collected about 20 to 30 kg of different size of mussels. Local dealers and agents are purchasing mussels at rate of Rs. 70 to 100 per kilogram and supplies to companies at rate of about Rs.130 per kg. The companies are properly, washing, cleaning, processing and packing mussels in live form according to international standards. Very fresh and hygiene live green mussels exporting shipments about 1200 to 2000 kg to international market on daily basis. High tides monsoon rains and flooding seasons are effect the natural catch and production of green mussels. No any artificial culturing system is so far observed at Coastal zone of Sindh. The artificial propagation and culture is possible through raft system, the seed may be collected from natural spawning grounds and mudflats. The pollution, freshwater scarcity, over exploitation and undersize catch is a major cause of reduction the population, diversity and production of green mussels. It is need of possible capacity building of harvesters, sustainability in catch and conservation process for existences of green mussels.

DNA BARCODE STUDY FOR THE TAXONOMIC IDENTIFICATION OF GENUS *ILYOPLAX* (OCYPODOIDEA, DOTILLIDAE) FROM INTERTIDAL AREAS OF PAKISTAN

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Most of the crab species inhabiting the North Arabian coast remain unidentified or poorly defined as have been characterized morphologically but not genetically. In the current research, crabs of genus *Ilyoplax* were collected from the mangrove ecosystems along the Pakistan coast has been carried out. These crabs are intertidal, semiterrestrial and live in allied areas of the mangroves; thus have a significant role in detritus formation, nutrient recycling and dynamics of the ecosystem. To clarify the genetic identity, DNA barcoding of sand bubble crabs through the sequencing of partial mitochondrial COI gene were analysed. In addition to molecular identification and confirmation, the key morphological characteristics were also assessed. DNA barcoding of partial mitochondrial COI gene sequences confirmed the identification of 3 genetically isolated species i.e. *Ilyoplax frater*, *I. stevensi* and *I. sindensis* (Previously identified as *I. species*) through the Neighbor joining and Maximum Likelihood method. The nucleotide frequencies were moved toward the A-T base pair (52.5% to 62.5%) whereas interspecific divergence ranged from 6.4% to 10.5%. DNA barcoding with precise morphological identification was effective to characterizing the crab species collected from the coast of Pakistan. Our study confirms the application of DNA barcodes database as highly suitable and effective identification system for the analyzed marine crustaceans.

GENETIC DIFFERENTIATION OF HERMIT CRAB OF SUPERFAMILY PAGUROIDEA FOUND ALONG THE COAST OF PAKISTAN: A BIOCHEMICAL APPROACH

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Hermit crabs (Decapoda: Crustacea) are typical marine organism mostly distributed on muddy, rocky and sandy habitats of coastal areas of Pakistan. They play a dynamic role in the marine ecosystem as the cleaner and an important role in community structure. The major reported genera includes; *Pagurus*, *Paguristes*, *Calcinus*, *Diogenes*, *Dardanus*, *Clibanarius*, and *Coenobita*, which belong to the family Paguridae, Diogenidae and Coenobitidae. The ponderable aim of this study was to investigate the genetic and biochemical analysis of superfamily Paguroidea (10 species) occurred abundantly and collected from the different coastal areas of Pakistan. Total three isoenzymes [Catalase (CAT), Amylase (AMY) and Carbonate dehydrogenase (CD)] were examined in all hermit crab species to analyze the level of differentiation among the species of the superfamily Paguroidea through polyacrylamide gel electrophoresis (vertical) PAGE. Percent polymorphic loci (*P*) found higher in *Clibanarius signatus* (*P* = 65.22%) and was observed lowest (*P* = 34.78%) in *C. infraspinatus*. Isozyme variations revealed the two heterozygous loci (*CD*-1* and *CD*-2*) in *Diogenes planimanus* and only one (*CD*-1*) in *Clibanarius signatus*. The mean expected heterozygosity was found higher (0.22 ± 0.34) in *C. signatus* and was observed lowest (0.20 ± 0.15) in *D. planimanus*. The current study also determines that *D. planimanus* presented significant biochemical variations and further studies are now in process on an advanced level (DNA markers) to resolve the similarity that likely due to the presence of two species or sub-species.

STUDIES ON GENETIC VARIABILITY IN COMPARISON WITH MORPHOLOGY FROM FOUR POPULATIONS OF COMMERCIALY IMPORTANT SHRIMPS *FENNEROPENAEUS INDICUS* (BRACHYURA: DECAPODA: PAENIDAE) FOUND IN COASTAL WATERS OF PAKISTAN

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The isozyme and morphometric variations were investigated in four populations of commercially important white shrimp (*Fenneropenaeus indicus*). The white shrimps were collected from the Korangi fish harbor, Karachi

Fish Harbor, Ketibunder and Sonmiani. The total polymorphic loci percentage varied in all five investigated isozymes i.e., Catalase (CAT), Carbonic anhydrase (CA), Creatinine kinase (CK), Peroxidase (PRX) and Amylase (AMY). The AMY represented 100% polymorphic loci, CAT and CA exhibited 66.66% polymorphisms, whereas, CK and PRX revealed 0% polymorphism. The overall frequency of polymorphic loci was found 0.66 in the populations of Ketibandar and Korangi fish harbors. Morphometric analysis (carapace length, abdominal length, total+ length, wet weight) of both genders was performed to evaluate the condition factor (CF). The highest CF (0.5473) assessed in the populations of Karachi fish harbor. The results revealed the significant variations among the population of white shrimp.

**MOLECULAR IDENTIFICATION OF PLEUSTONIC SIPHONOPHORE, *PHYSALIA PHYSALIS*
(HYDROZOA: PHYSALIIDAE) (PORTUGUESE MAN O' WAR) FROM THE SANDSPIT,
COAST KARACHI, PAKISTAN**

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Physalia Physalis Linnæus, 1758, (Portuguese Man o' War) or blue bottle is the single genus of the family Physaliidae and distributed in the tropical to temperate waters throughout the world including Indian, Pacific and Atlantic Oceans. Bright blue-purple colonies of *P. physalis* have distinctive morphological characteristics; an obvious pneumatophore, and thin, ribbon-like long tentacles bearing numerous nematocysts. The *P. physalis* feeds on fishes and Shellfish whereas severe invasion of this species resulted the reduction of fisheries. *P. physalis* colonies washed ashore during the Southwest monsoon in the Sandspit coastal area of Karachi, Pakistan. The colonies of *Physalia physalis* collected from the Sandspit. The identification and confirmation of *P. physalis* based coding region of mt-DNA Cytochrome Oxidase I (COI) gene. *P. physalis* showed the 96% sequence similarity to the gene sequence of *P. physalis* from the USA by using the online NCBI Blast tool. Genetic divergence and the evolutionary relationship also estimated using the Maximum Likelihood method based on the Tamura-Nei model.

**SEASONAL BIOCHEMICAL CONSTITUENTS VARIATION IN MUSCLES OF TWO SEA SNAKES
(*HYDROPHIS CAERULESCENS* AND *H. SCHISTOSUS*) FOUND IN COASTAL WATERS OF
SONMIANI (DAMB), BALUCHISTAN, PAKISTAN**

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Sea Snakes mainly adapt to the marine environment and they occur in tropical and subtropical regions. They found along the coasts, creeks, inland and as well as from the sea (more than 100 miles). About 90 species of sea snakes are reported in all over the world and 14 species were reported in Pakistani waters. The current study aimed to evaluate the total concentration of protein, carbohydrate and lipid in the tissues of two sea snake species (*Hydrophis caeruleus* and *H. schistosus*) collected in pre-monsoon (PRM) and post-monsoon (POM) seasons from the coast of Sonmiani (Damb) Balochistan. The variations were observed in biochemical constituents between the species and as well as between the seasons. In *H. caeruleus*, the highest level of protein (51.87%) was present, followed by carbohydrate (7.15%) and lipid (3.79%) during PRM. Similarly, the highest protein (41.39%) found in *H. schistosus*, whereas carbohydrate (4.36%) and lipid (2.85%) observed in low quantity. The biochemical constituents showed low levels in biochemical component during POM in the both species as compared to PRM. The significant difference was observed in the percent occurrence of protein as was observed (2.99%) in *H. caeruleus* and (3.85%) in *H. schistosus*. This study revealed the significant seasonal variations in biochemical aspects, especially in protein levels of the two species; the further studies required to identify the causes of biochemical changes in coastal waters of Pakistan.

FIRST RECORD OF POLYCHAETE *SIGAMBRA PETTIBONEAE* HARTMANN-SCHRÖDER, 1979 AND *S. TENTACULATA* (TREADWELL, 1941) (FAMILY PILARGIDAE) FROM THE COAST OF PAKISTAN

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Family Pilargidae Saint-Joseph, 1899 is reported for the first time from Pakistan. Twenty six specimens of *Sigambra pettiboneae* Hartmann-Schröder, 1979 and four specimens of *S. tentaculata* (Treadwell, 1941) were collected from intertidal zone of Korangi Creek, Karachi, Pakistan. *S. pettiboneae* differs from *S. tentaculata* in having curved notopodial hook from 8th chaetegerous segment instead of 4th chaetegerous segment. *S. tentaculata* closely resembles with *S. parva* (Day, 1963), reported from South Africa, in having curved notopodial hook from 4th chaetegerous segment but differs in the number and shape of the pharyngeal papillae. Detailed description and illustrations of *S. pettiboneae* and *S. tentaculata* based on Pakistani materials are given herein.

THE STUDY OF REPRODUCTIVE BIOLOGY OF *SIPHONARIA KURRACHENSIS* FROM THE ROCKY COASTS OF KARACHI

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Siphonaria is a genus of air-breathing sea snails or false limpets, marine pulmonate gastropod molluscs in the family Siphonariidae, the false limpets. This genus occurs worldwide in most tropical and temperate seas. The specimens of *S. kurracheensis* on histological examination were grouped as pure male, ambisexual, predominantly females and predominantly males. The spawning in *S. kurracheensis* was found during summer-early winter at Mubarak Village. The spawning of the same species in different seasons at different sites may be linked to availability of food. Similarly, the reproductive cycle has been linked to food availability in *S. capensis* and *S. serrata* (Pal & Hodgson, 2005) in *S. diemenensis* (Quinn, 1988b) in *P. japonica* (Liu, 1994) in *Cellana tramoserica* (Fletcher, 1984 a & b) and in *P. granularis* (Bosman & Hockey, 1988).

SIZE AT SEXUAL MATURITY AND FEUNDITY OF THE BLUE SWIMMING CRAB, *PORTUNUS PELAGICUS* (LINNAEUS, 1758) FROM DAM SONMIANI AT BALOCHISTAN COAST, PAKISTAN

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This paper deals the size at sexual maturity and fecundity of the blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758). Size a sexual maturity attained by the male and female crabs of *P. pelagicus* was determined by relative growth (functional) as well as by examining the condition of gonads (physiological). During the study it was observed that the physiological and functional maturities occur almost at the same size which was from 69 to 73 mm short carapace width or 82 to 91 mm long carapace width. Male *P. pelagicus* matures earlier than the females. The size at which females attains full sexual maturity is 73-82 mm short carapace width. The 50% population of male and female crabs attains sexual maturity at 63.57 mm and 83.55 mm short carapace width, respectively. The minimum number of eggs was 86392 in a crab of 73 mm short carapace width whereas maximum number of eggs was found to be 1555820 in a crab of 138 mm short carapace width. Fecundity was significantly co-related to crab size, which

larger crabs producing a greater number of eggs. The average fecundity was 393146.3 ± 307051.7 (S.D) for a berried crab with a mean short carapace width of 98.53 ± 18.05 .

REARING, DISTRIBUTION, MARKETING OF CORALS OF KARACHI COAST, PAKISTAN

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This study is objecting the some of the early studies on coral research in Pakistan. These studies are poorly reporting corals and acclaiming that there is no proper habitat for corals along the Karachi Coast. But the author found that water current and clarity at Rehman Goath (French Beach) and Pacha Bnder are ideal for coral park in Pakistan. 95% of the corals are distributed at non tidal zones of Karachi. The author founds more than 15 species of coral at Do Derya, Manora, Kaka Village, Sands spit, Rehman Goath, Buleji, Pacha Bundar, Light house, Mubarak Goath Pitkori and Sonera (extreme West Coast). The author is rearing LPS and SBS coral in captivity since 20 years. It is found that water circulation is the only key which a common hobbyist can also adopt by using the submersible pump. Provided that there should be no mettle nuts, bolts, etc. to be attached at the submersible pump. Other instrument like protein, skimmer, chiller, heater, ozonizes, UV filter, etc. make it easier to rear corals. Moreover, dozing of calcium chloride/calcium bi-carbonate is also suppose plus point to rear corals. This dozing also enhance the growth of coralline algae in marine aquarium Corals are supposed ban to trade all over the world. But some companies are having license to market .5-6 importer of corals are also working in Pakistan. They are collecting highly beauty full corals that attracts every common man. The import acken corals of 5-6 inch diameter in having whole sale price of Rs.4,000/= and local is having price of Rs.1000/=. The flower pot imported of 2 inches was having price of Rs.6000/= and local of Rs. 300/= etc.

STUDY OF BARCODE DATA TO RESOLVE THE SPECIES OF GENUS TUBUCA ALONG THE COAST OF PAKISTAN

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Preceding study has recognised the inter-population morphological variability among the species of genus *Tubuca*. To determine the source of inter-population variation (genetic or environmental) of each species of genus, morphological and genetic data were investigated from crabs collected from 4 sites (Sandspit, Korangi, Sonari and Sonmiani) along the coast of the Pakistan. The DNA barcode study aims to provide an efficient method for species-level identifications and, as such, will contribute powerfully to taxonomic and biodiversity research. The discrimination of species in the genus *Tubuca* is particularly difficult due to the overlap of morphological characters. Fiddler crabs are commonly found along the coastal backwaters (mangrove swamp) of Pakistan. Previously, no research has been done based on barcode data, but more work has been done on taxonomic identification on this genus in the world so far. In this study, we used the 5' (barcode) and 3' regions of cytochrome oxidase I (COI) to test their utility in the identification of species in this genus as well as closely related species. Both regions were useful to discriminate all the species tested. However, the non-barcode 3' region resulted in higher resolution and support for species relationships when the data were analyzed using both Maximum Likelihood and MrBayes. Genetic distance also showed higher level of variation between two species (2.5%) using the 3' region of COI that showed higher level of intra-specific variation in isozyme (37% polymorphic loci) and molecular data (genetic distance = 0.7%) which suggested the need of further analysis from different localities and use of different molecular markers to resolve this issue.

4. PALAEONTOLOGY

GIRAFFOKERYX AND GIRAFFA (RUMINANTIA, GIRAFFIDAE) FROM KUND LOCALITY OF LOWER SIWALIKS, PAKISTAN

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Giraffokeryx punjabiensis and *Giraffa priscilla* have been reported from the outcrops of Kund, district Chakwal, Punjab, Pakistan. This Middle Miocene locality (ca. 14.2–11.2 Ma) is located about 3.5 km south east of the Chinji village (Lat. 32° 68' N, Long. 72° 40' E) in the Lower Siwalik, northern Pakistan. The material comprises isolated teeth, maxilla and mandible fragments which shows some primitive features for the Lower Siwalik giraffids. The lower cheek teeth are quadrate with a very weak stylids, median ribs and ectosylids. The lower sized giraffids preferred to inhabit wooded mean forested areas of the Siwalik Group. The giraffids have been recovered from this area for the first time.

RHINOCEROTIDS (PERISSODACTYLA) FROM MIDDLE SIWALIKS OF NORTHERN PAKISTAN

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New dental material of Rhinocerotids from the Middle Siwalik deposits of Potwar Plateau, Punjab, Pakistan has been described. The fossiliferous sites, Dhok Pathan of Pathan Formation (Late Miocene – Early Pliocene) and Kundal Nala of Nagri Formation (Early-Late Miocene) located in district Chakwal, while Hasnot and Padhri of Dhok Pathan Formation are in district Jhelum, Punjab, Pakistan. The recovered material comprises isolated premolars and molars. The two identified species of Rhinocerotids include *Chilotherium* cf. *intermedium* and *Alicornops* sp. These specimens provide additional information about the recorded species and contribute to recent work of Perissodactyla from the Middle Siwalik Hills of Pakistan.

STEGOLOPHODON (PROBOSCIDEA, MAMMALIA) FROM SIWALIK GROUP, PUNJAB, PAKISTAN TETRACONODON AND SIVACHOERUS (SUIDAE) FROM POTWAR PLATEAU, NORTHERN PAKISTAN

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Rare tetraconodonts, *Tetraconodon magnus* and *Sivachoerus prior* are ascribed in this article. *Tetraconodon* is believed to be occurred in the Upper Dhok Pathan and Tatrot formations of the Siwalik Group. However, the specimens described here from the mid Dhok Pathan Formation are important in relieving the long-held notion of the previous researchers. Similarly, *Sivachoerus prior* appeared earlier than it was thought previously.

CHOEROLOPHODON FROM THE MIDDLE SIWALIKS OF PAKISTAN

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Monogeneric family Choerolophodontidae, has long been considered Subfamily of Gomphotheriidae, Proboscidea. Only a single species, *Choerolophodon corrugatus*, has been recorded from the Siwaliks and it is predominantly Middle Siwalik species. The newly discovered specimens, including the tusk fragments, isolated upper and lower deciduous premolars and permanent molars, show great insight of this species. The cheek teeth are quite similar in general outlines to European and African species of the genus but mandibles are different in some species. Also, there are considerable variations in the cementodontology and choerodontology of the studied specimens indicating the possibility of a new species in the Siwaliks.

**DESCRIPTION OF BOSELAPHINE REMAINS FROM THE LATE MIOCENE
SIWALIKS OF HASNOT, PAKISTAN**

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Current study focuses on the morphometric analysis of Boselaphine remains belonging to genera *Pachyportax* and *Selenoportax*. The available information on both taxa is scanty and only few specimens are known for both genera especially *Pachyportax*. The members of both genera have medium to large sized body with strong hypsodonty of teeth representing grazing on coarse grasses and shrubs in the late Miocene Siwalik habitats of Potwar Plateau. At the apex of molar, crown is narrow and represents selenodonty in *Selenoportax*, while a broader crown in *Pachyportax* is indicative of strong hypsodonty. The currently discovered samples may add additional information on dental morphology and dietary habits of these extinct boselaphine taxa which are represented today by their living kin *Boselaphus tragocamelus*.

NEW BOVIDS FOSSILS FROM JHELM TATROT FORMATION, PUNJAB, PAKISTAN

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Four species of Family Bovidae have been described and discussed from the Tatrot Formation of Pakistan. The Tatrot Formation is the part of Upper Siwalik Subgroup and is overlying on the Dhok Pathan Formation of the Middle Siwaliks. The specimens include horn core fragments, mandible fragments, and isolated lower premolar and molars, belonging to *Kobus porrecticornis*, *Sivacobus* cf. *patulicornis*, *Reduncini* sp. indet. and *Boselaphus* cf. *namadicus*. These species belong to the tribes Reduncini and Boselaphini. *Boselaphus* cf. *namadicus* is considered the closely related or direct ancestor of the living species *Boselaphus tragocamelus*.

TRAGOPORTAX SALMONTANUS (BOVIDAE, MAMMALIA) FROM THE MIDDLE MIOCENE OF PAKISTAN

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Tragoportax salmontanus have been recovered from the Chinji Formation of the Lower Siwalik Subgroup, Pakistan. The remains comprise horncores, maxillary and mandibular fragments, and isolated teeth. Having compared with the earlier described bovid remains from the Siwaliks, the new material has been allocated to *Tragoportax salmontanus*. This species is well represented in the Middle Siwaliks but has not been previously reported from the middle Miocene Chinji Formation of the Siwalik Group. We report *T. salmontanus* from the middle Miocene Chinji Formation of Pakistan, resulting extend the time range for the species from the middle Miocene to the late Miocene of Pakistan.

NEW BOVID REMAINS (GAZELLA SP.) FROM MIDDLE MIOCENE OF THE SIWALIKS OF NORTHERN PAKISTAN

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New remains of *Gazella* sp. are recovered from the Siwalik middle Miocene of Pakistan. The worth describing five specimens are sorted out for the taxonomic study. The specimens belong to *Gazella* sp. These specimens are collected from the middle Miocene locality of Dhok Bun Amir Khatoon from the Chinji Formation of the Siwalik Group. The locality age is from 14.2 Ma to 11.2 Ma. The defined specimens include isolated molars and left mandibular fragments. The specimens are recognized based on morphometric assessment with formerly known fossil material from the Dhok Bun Amir Khatoon outcrops. *Gazella* sp. has been recovered only from the middle Miocene (14.2-11.2 Ma). *Gazella* sp. is a rare taxon recorded from the Lower Siwalik Subgroup.

NEW REMAINS OF BOVIDS (MAMMALIA, BOVIDAE) FROM THE CHINJI FORMATION OF CHABBAR SYEDAN (MIDDLE MIOCENE), PAKISTAN

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This research describes new fossils of bovids originating from Chabbar Syedan, a long neglected Siwalik locality of northern Pakistan. Bovid fauna recovered in the present study comprised of *Miotragocerus gluten*, *Sivoreas eremite*, *Sivaceros gradiens*, *Helicoportex praecox*, *Eotragus* sp. and *Gazella* sp. The studied material comprises of about 40 specimens including maxillae, mandibles, isolated premolars, molars and horn cores. The faunal composition of Chabbar Syedan locality suggests a Middle Miocene age and it belongs to Chinji Formation. The commonness of the bovid remains at studied section designates a large amount of the bovid communities at the time of stratification like other Middle Miocene localities of Siwaliks.

NEW DENTAL REMAINS OF *PERCROCUTA* (CRANIVORA: MAMMALIA) FROM THE SIWALIKS OF NORTHERN PAKISTAN

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New specimens of hyaenids, *Percrocuta carnifex* were collected from the late Miocene of the Siwaliks of the Northern Pakistan. Dental remains contain well preserved canines and lower premolars and molars. The carnivores are rare in the Siwaliks. This study gives evidence about the community relations (prey and predatory relationships) of the Siwaliks at that time.

SOME NEW FOSSILS OF *GIRAFFOKERYX PUNJABIENSIS*, FROM DHOK BUN AMEER KHATOON LATE MIOCENE, SIWALIKS, PAKISTAN

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Fossil site Dhok Bun Ameer Khatoon yielded some assemblage of even toed mammalian remains of family Giraffidae of the middle Miocene. This site has well exposed Chinji and Nagri formation. The material comprises predominantly isolated teeth. The well preserved upper and lower dentition allows the presence of *Giraffokeryx* cf. *punjabiensis* in the Chinji Formation of the Lower Siwaliks. Palaeoenvironmental conditions suggested that Miocene climate of Pakistan was most likely to be monsoonal as there is now a days. The presence of giraffids suggests woodland habitat with swamps in the Dhok Bun Ameer Khatoon and its surroundings.

DEPLETION OF WATER BODIES WAS AN ECOLOGICAL STRESS FOR EXTINCT AMPHIBIOUS MAMMALS: ENAMEL HYPOPLASIA BASED EVIDENCE

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Enamel hypoplasia is a tooth malady resulted as depletion of enamel forming cells called ameloblast. It is a reliable and permanent marker for the ecological stresses in the life history of a mammal. In this study the dental remains of *Hexaprotodon sivalensis* (an extinct species of hippopotamids) from late Miocene to late Pliocene outcrops of the Siwaliks of Pakistan were analysed for occurrence of enamel hypoplasia to trace out the impact of water resources reduction on these amphibious mammals. The occurrence of enamel hypoplasia is significantly high $p > 0.05$ in the Siwalik *H. sivalensis* during Upper late Pliocene interval that indicates a high level of ecological stress faced by *H. sivalensis* during Upper late Pliocene interval than the late Miocene-Lower late Pliocene interval. Different authors had reported dry environmental condition after 09 Ma and there was increase in the aridity during Pliocene. The glaciation during late Pliocene had also added up in reduction of fresh water bodies during this time period so this comparatively high level of stress in the Siwalik hippopotamids of late Pliocene traced out by current enamel hypoplasia analysis might be due to the depletion of water bodies during this geological interval.

DESCRIPTION OF THE NEW DENTAL REMAINS OF SUIDS FROM THE MIDDLE MIOCENE SIWALIKS OF PAKISTAN

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The fossil collection was made from the Middle Miocene deposits of the Siwalik of Pakistan (Chinji Formation). The collected fossil material is identified as suid species named as *Listriodon pentapotamiae* and *Conohyus sindiensis*. Both species correlate the Asian and European fauna due to close resemblance of morphometric characters. *Listriodon pentapotamiae* relates European *Listriodon splendens* and Asian *Listriodon mongoliensis*. Lophodont molars correlates the *Listriodon* evolution with the modern suid groups. *Conohyus sindiensis* is considered as the evolutionary derivative of genus *Palaeochoerus*. All these information tell us about the different aspects of the suid species that were one living in the Middle Miocene Siwalik ecosystems.

NEW DENTAL REMAINS OF *DORCATHERIUM* (ARTIODACTYLA, TRAGULIDAE) FROM THE MIDDLE MIOCENE SIWALIKS OF PAKISTAN

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The newly discovered dental remains included in this study were collected from the Chinji Formation of the Siwaliks of Punjab, Pakistan that had a chronological age of 14.2-11.2 Ma. The comparative morphometric analysis of these isolated premolars and molars with available literature has attributed these remains to two species named as *Dorcatherium majus* and *Dorcatherium minus*. *Dorcatherium* is a predominant Middle Miocene Siwalik genus of family Tragulidae. Tragulids are the most primitive representatives of the extant ruminants. Tragulids of Southern Asia were species rich during Middle Miocene but their diversity reduced after 07 Myrs. This systematic study of the discovered remains strengthens the available information about the Middle Miocene Siwalik Tragulids.

SYSTEMATIC STUDY OF THE *EQUUS SIVALENSIS* (PERISSODACTYLA, EQUIDAE) DENTAL REMAINS FROM THE SIWALIKS OF PAKISTAN

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The systematic studies of dental remains that are newly discovered from the Pabbi Hills (Upper Siwaliks) of Pakistan have showed that these remains have dental features resemble with extinct equids. In these remains the protocone of the molars are not isolated that differentiate these remains from Hipparion. Further differential diagnosis has attributed these remains to *Equus sivalensis*. This species is also reported from the Upper Siwaliks of India that indicates the faunal affinities in the Upper Siwaliks of Pakistan and India. The lower molars are relatively small sized and narrow having thin enamel with shallow ectoflexid and metastylid and have V-shape lingual depression with well-developed plicaballoid that is more directed at posterior side in *Equus sivalensis* as compared to other species of *Equus*. The measurement of crown height shows that this species is hypsodont in nature.

**NEW FOSSIL COLLECTION OF *HIPPOHYUS SIVALENSIS* (ARTIODACTYLA: SUIDAE: SUINAE)
FROM LATE MIOCENE TO PLIOCENE OF SIWALIKS OF PAKISTAN**

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Hippohyus sivalensis is a common suid of late Miocene to Pliocene rocks of Tatrot/Hasnot area of Pakistan. The molar resemblance with equids indicate their grazing feeding habits. This species migrated to Potwar land when grassland established there. It has typical suine characters with hypsodont dentition. The described material consists of isolated molars. This discovery will provide a new insight to understand the diversity and geographic distribution of Siwalik Suids.

5. WILDLIFE, DIVERSITY AND CONSERVATION

INTRASPECIFIC BIODIVERSITY OF ROSE-RINGED PARAKEET (*PSITTACULA KRAMERI*) OF DISTRICTS HYDERABAD AND LARKANA, SINDH, PAKISTAN

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Psittacula krameri belongs to the Phylum Chordata, Class Aves, Order Psittaciformes, Family Psittaculidae and Genus *Psittacula*. Parrots are the tropical birds inhabiting trees of the warm climates and possess many apical characteristics similar to the crows. They are the most intelligent birds and certainly possess more memory power. They are predominantly vegetarian and they make use of the beak for breaking the hard shells Young. During the course of present studies 12 species of *Psittacula krameri* were collected which include 03 ♀♀ and 03 ♂♂ from District Larkana and 03 ♀♀ and 03 ♂♂ from District Hyderabad. These species reflect diversification in different parameters such as: weight of the body; length of entire body; length of the wing cord; length of the wing feathers; length of the coverlet; length of tarsal; length of beak; length of tail feathers; length of hind foot; no. of wing feathers; no. of coverlets and no. of tail feathers respectively.

ROLE OF DNA MINI-BARCODING TECHNIQUE IN CONTROLLING ILLEGAL PRODUCT MANUFACTURING AND EXPORTATION FROM WILDLIFE SKIN

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Illegal trade is a major threat to the biodiversity and the efforts initiated for the conservation of wildlife. The shortcomings of the traditional taxonomic identification methods have been coped by a revolutionary and emerging technique, the “DNA barcoding”. Here we report a case of trader who was allegedly making footwear for a famous international celebrity from wild animal cutis. The samples confiscated during a raid on a footwear manufacturing industry by KP Wildlife department in August, 2016, were received by Bioresource Research Centre (BRC), Islamabad, for molecular identification on. The samples identified via DNA mini-barcoding by targeting *cytochrome oxidase I (COI)* gene belong to *Gazella bennettii* and *Bos taurus*. Such studies are helpful for credible investigations that only lead to effective prosecution and control of illegal wildlife trade ultimately helping in conservation of wild animal.

COMMUNITY BASED SNAKE CONSERVATION, LEAPING FROM AWARENESS TO ACTION IN LESSER HIMALAYAS AZAD KASHMIR PAKISTAN

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Natural ecosystems are facing a rapid decline of biodiversity around the globe, which has critical implications on ecosystem functions and services. Successful conservation efforts to slow this decline rely on the ability to monitor species and understand their ecological role. Such efforts are often hindered by a lack of knowledge

regarding arcane interactions. Snakes provide several key regulating and supporting ecosystem services, including insect suppression and nutrient cycling. However, trophic interactions that occur in both natural and anthropogenically impacted systems remain largely obscured. Exploring the diversity, diet, and anthropogenic pressure of snake distributions along elevation gradient and diet can also aid in the understanding of their foraging ecology, which will guide future management. In territories of Azad Kashmir (Pakistan), snakes are represented by 25 species and are facing anthropogenic, climatic, and natural predator pressures. The present study was conducted in azad Kashmir of lesser Himalayas to record mortality of snakes by anthropogenic pressure in villages, streams, road kills and natural predators during summer season. We estimated 500 snakes are killed in area of 40 km². The most killed species are, oriental rat snake (*Ptyas mucosa*), diadem snake (*Spalerosophis diadema*) and least killed are venomous snakes. The venomous snakes (*Bungarus caeruleus*, *Naja oxina*, *Echis carinatus*, *Agkistrodon himalayanus*) are killed by shepherd during harvesting of grasses. All the killed species are due to the superstitious behavior of uneducated humans and also are causing significant losses in snake populations. We have to plan for conservation, by grass root (school level to university) and community based professional training, by emerging methodologies and technologies to provide new windows into these otherwise opaque questions.

**BIOLOGICAL STATUS, DISTRIBUTION AND MAJOR THREATS TO THE WILDLIFE SPECIES
(VERTEBRATE FAUNA) OF SHEIKH BADDIN NATIONAL PARK KHYBER
PAKHTUNKHWA, PAKISTAN**

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The wildlife survey in Sheikh Badin national park (SBNP) was conducted from September, 2017 to August, 2018, to establish baseline information about existing wildlife “vertebrate fauna” in order to make some vantage point for future monitoring of key wildlife “vertebrate species”. Using different direct and indirect investigation techniques, various parts at the unlike elevation of Sheikh Badin national park (SBNP) were surveyed and observation on various animal and their habitats were recorded. The study was conducted with aim to know the biological status, distribution and threats to the vertebrate fauna of Sheikh Badin national park. The survey at the different elevation leads us to the presence of different vertebrate animals, So far, the result shows the presence of Jackals (*Canis aureus*), Wolf (*Canis lupus*), Fox (*Canis vulpus*), Jungle cat (*Felis chaus*), Rat (*Rattus rattus*), Rock pigeon (*Columbia liva*), Black partridges (*Francolinus francolinus*), chukor (*Electoris chukor*), Broun partridges (*Francolinus pondiceranus*). The population of these wildlife species is very low with respect to potential habitat available in this area. Threats to wild life vertebrate species were found the illegal hunting, habitat destruction and other natural as well as human activities destroy the population of vertebrate species in the area. This study played an important role in the awareness of local community through various methods for the conservation of vertebrate species in SBNP. The study also reports to the wildlife conservators and high officials that habitat protection is suggested to be very helpful in the conservation of vertebrate species in Sheikh Badin national park district D.I Khan Khyber Pakhtunkhwa Pakistan.

**HABITAT SELECTION OF COMMON MOORHEN (*GALLINULA CHLOROPUS*) DURING BREEDING
IN IMPOUNDED MARSH WETLANDS IN DISTRICT BAHAWALPUR, PUNJAB**

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This study has provided basic insight in to the habitat selection of common Moorhen during breeding in the area of Bahawalpur at different locations. First of all vegetation survey was conducted and the flora species of *Acacia nilotica*, *Prosopis juliflora*, *Dalbergia sissoo*, *Typha elephantiana*, *Cynodon dactylon*, *Saccharum bengalensis*,

Solanum nigrum, *Calotropis procera*, *Phoenix dactylifera* and *Zizyphus numularia* were recorded which were most commonly present in the habitat of moorhen. *Typha elephantiana* was the most abundant and preferred habitat for moorhen during breeding season. Moorhen nests were searched through visiting all possible sites and monitoring the activities of the adults at dawn and dusk. Moorhen only used the microsites to build nests on the branches of trees or emergent vegetation bended on water up to 3-5 feet above the water level. These birds used foliage of *Dalbergia sissoo*, *Prosopis juliflora* and *Acacia nilotica* and other nearby vegetation as hide. Soil and water samples were also analyzed for selected habitat of moorhen. Data obtained is a major addition in existing meager information on common Moorhen, its habitat use, breeding biology preferred by this species in its habitat.

BEHAVIOR AND FEEDING ACTIVITIES OF WILD ANIMALS IN BAHAWALPUR ZOO, PUNJAB, PAKISTAN

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Current study was conducted on behavior and feeding activities of wild animals present in Bahawalpur Zoo. The main objective of the study was to analyze the effect of captivity on the feeding and behavior activities of wild animals present at zoo. Activities like active, passive and abnormal were observed during the study. Observations were taken on morning and evening basis specially at the time on which diet was provided, amount of diet, food presentation and dispersal ways, animal's preference for food, induction of novel objects, presence of any feeding enrichment technique, hygienic conditions regarding food given and enclosures. Various stereotypic behaviors have been reported like self-aggression, head tossing and pacing. Such types of behavior were most commonly observed in Lion and Puma. Baldness and head tossing in one of the bears was observed that is the result of poor animal welfare. Unhygienic conditions in lion's enclosures were also observed. Food provided to the animals like Desert cat, Jackal, Asiatic cheetah, Indian wolf and Puma is less than the per day caloric requirement of the animals. Habitat management, feeding enrichment and the proper medications would be the best solutions to improve habituation under captivity. It will also increase the rate of reproduction in animals.

DISTRIBUTION AND DIET COMPOSITION OF GREY WOLF (*Canis lupus*) IN MAHOODAND VALLEY, SWAT

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Grey wolf (*Canis lupus*), is one of the largest species of Canid family, occurring in various parts of the country including upper Swat (Matiltan and Mhoodand areas). This species is categorized as "Endangered" in Pakistan and reportedly, its population is declining in its range. Scientific data on ecological aspects of the species are scanty in the country, therefore, the current study aimed at focusing on some of the ecological parameters of the species including its distribution, population, food habits and human-wolf conflict in Mahoodand area, Swat. The distribution of grey wolf in the study area was determined by conducting field visits recording its direct and indirect signs including scats, pug marks, foot prints and presence of dens in the area. Food habits of the species were investigated by analysis of its scats samples, while human-wolf conflict was investigated by collecting data from native people using self-designed questionnaires. The grey wolf was recorded and found distributed at eleven out of 18 sampling sites surveyed. The elevation range of its distribution was between 2332 m to 2926 m above sea level (ASL). A total of 7 dens, 30 pug marks and 33 scats of grey wolf were recorded at 18 different sampling sites in the study area. Scat analysis of the species showed 5 domestic and 8 wild prey species in its diet menu, with 53.66% contribution from

livestock and 46.34% from the wild prey. The grey wolf was found as one of the major predators in the study area consuming mainly donkey, horse, cow, sheep and goats. Most of the depredation occurred at night while maximum livestock were killed in winter season. The current study concludes that the Matiltan Mahoodand area is an important area regarding distribution of grey wolf, however. Human-wolf conflict demands urgent conservation measures to be launched in the study area in order to save the population of this top predator in the area.

DISTRIBUTION AND DIET COMPOSITION OF ASIATIC BLACK BEAR (*URSUS THIBETANUS*) IN BAJAUR AGENCY, PAKISTAN

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Asiatic black bear (*Ursus thibetanus*), a member of family Ursidae, occurs in different parts of the country including Bajaur Agency. The species is categorized as “Vulnerable” and its population is reportedly declining due to many factors. In the present study, we investigated its distribution and diet composition in Bajaur Agency, Pakistan. Distribution of the species in the study area was studied by conducting field surveys at potential sites and recording its direct and indirect signs such as scats, pug-marks, dens, dead remains, and scratches on plants stems. Diet composition was investigated by analyzing scats of the species which were collected from the study area. Asiatic Black Bear was recorded and found distributed at different sites in three out of four Tehsils surveyed, while one site was found negative regarding occurrence of the species. The elevation range of its occurrence was from 792m to 2286m. A total of 7 different signs were recorded including scats (03), pug-marks (02), one scratch on stem of plant and one dead remain. Maximum signs were recorded in Tehsil Khar. Scat analysis revealed both plant-based as well as animal-based diet of the species, with greater contribution from the plant food. A total of 10 different plant species were recorded in its scats with major contribution from Maize. The animal prey species of Asiatic black bear identified in the scats included insects, hare, domestic goat, sheep and cow.

DIVERSITY AND ABUNDANCE OF AVIFAUNA IN MANGLOT WILDLIFE PARK, DISTRICT NOWSHEHRA

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Comprising about 13% of the world avian diversity, Indian sub-continent has approximately 1300 species of birds. The assessment and evaluation of bird communities is considered as an important tool in biodiversity conservation efforts. There are more than nine thousand species of birds existing globally belonging to twenty nine orders. In Pakistan, there are 660 species of birds belonging to twenty two orders. The abundance and diversity of avian species in a specific habitat could serve as a useful barometer of the ecological status of that habitat. It has been estimated that as a result of all land use changes, there may have been a loss of 20-25% of pre-agricultural bird numbers. Moreover, it is predicted that about 27- 44% bird species could be lost to agricultural expansion from Neolithic to 2050. Birds present some important services to agro-ecosystems including as biological control agents of insects and rodents, plant pollination, scavengers etc. The current study, therefore, was designed to investigate the diversity and abundance of the avifauna occurring in the Monglot Wildlife Park, district Nowshera, from September 2017 to July 2018. Surveillance survey was conducted in the study area to determine diversity and abundance of avifauna in the study area. The selected sampling sites were visited on fortnightly basis to collect data by using Point Count Method. Field observations were performed with 15 days interval. Visits were conducted at morning hours

(6:30 am to 9:30 am) and in the evening hours (3:30 pm to 6:30 pm). Shannon's Diversity Index (H') was used to analyze the collected data for species diversity and relative abundance. A total of 52 birds species belonging to 10 orders and 27 families were recorded in the study area during the present study, including both summer and winter visitors. The order Passeriformes was found the most abundant having 16 families and 31 species. Species richness was found greater in winter than in summer. Diversity index of Avifauna was also high in winter season Species richness of avifauna was recorded low in winter than summer season at site first while remain low in winter but high in summer at the second site. Species evenness was observed high in winter but low in summer at the study site three.

FIRST RECORD OF *MICROPHALUS TASMANIAE* SMITH, 1973 IN BLACK-CROWNED NIGHT HERON *NYCTICORAX NYCTICORAX* FROM HAMAL LAKE, SINDH, PAKISTAN

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A thorough investigation was carried on Black-Crowned Night Heron, *Nycticorax nycticorax* for helminth parasitic infections from 10.2016 to 09.2017. Twenty-four Black-Crowned Night Herons were dissected and all were found positive with various forms of helminths including trematodes and nematodes. After dissection and teasing of the visceral organs, the whole content was thoroughly investigated on stereo dissecting microscope 11 out of 24 herons were parasitized with 75 specimens of *Microphalus tasmaniae* Smith, 1973. The identification of the trematode parasite was done with help of keys, internet and supervisors. This species is first time recorded from Sindh, Pakistan.

SURVEILLANCE OF DISEASES IN WILD MAMMALS OF ZOO SAFARI LAHORE FOR THEIR BETTER CARE AND CONSERVATION

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In 20th century captive and semi-captive housing conditions are emerged as an important tool for conservation of wildlife. But these facilities are facing many challenges like infectious diseases and shocks for upkeep of wildlife. The study was planned to access the causes of death in wild mammals and to provide data regarding prevailing diseases for better care and conservation. Data regarding animal care, diseases and postmortem findings was collected by frequent visit of the site from 2016 to 2017. A total of 92 deaths of 11 mammal's species were recorded at site. Among them carnivores were *Panthera tigris* (endangered) and *Panthera leo* (vulnerable) while herbivores were *Gazelle benettii*, *Antelope cervicapra* (endangered), *Dama dama*, *Axis axis*, Muntjacs deer, *Hylaphus porcinus*, *Equus quagga*, *Ovis orientalis* (vulnerable), *Boselaphus tragocamelus*. In total 83.6% deaths were of herbivores while carnivores' death toll was 16.3% in two consecutive years. The herbivores' mortality was 81.6% and 76% in 2016 and 2017 respectively. In 2016 carnivores' mortality was 18.1% and 13.5%. in 2017. Gender wise mortality records showed that males' deaths were 57% while females were 42.8%. Age wise death records indicated that 86% was cubs while 13% was adult in carnivores. In herbivores fawns' death was 10.3% and 89% was for adults. Early age mortality was significantly high in carnivores. Highest 50.9% mortality was recorded in *Antelope cervicapra* during 2016 due to Malignant Catarrhal Fever. In 2017 highest 24.7% & 24.9% mortality rates were recorded in *Axis axis* and *Hylaphus porcinus* respectively due to hemorrhagic enteritis, traumatic injury, jackal and stray dogs' attacks,

respiratory distress and cardiac arrest. In carnivores causes of death were septicemia, anemia, gastroenteritis and heart diseases. Overall infectious diseases (viral/bacterial) were found to be major cause of deaths while shocks/stress were second in this regard. But lack of field staff, under developed veterinary facilities, improper record keeping, lack of animal's behavior information and lab tests facilities increases the problem many folds. It is concluded that better management and care is more suitable than treatment.

TO EXPLORE THE CURRENT POPULATION AND DISTRIBUTION OF MARKHOR IN CHITRAL

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Markhor (*Capra falconeri*) the national animal of Pakistan is considered endangered as well as endemic species globally. Therefore, the present study was conducted in 2018 to explore and estimate the current population and distribution status of Markhor in Chitral. Study area was divided into season and area wise observation in most protected areas Chitral Gol National park covered (7750) ha and Game Reserves (i) Tooshi Gol (1545) ha, (ii) Golen Gol (4970) ha and Community Game Reserves (i) Begusht (800) ha, (ii) Tooshi Shasha (20000) ha, (iii) Arkari (10000) ha, (iv) Gehirat (95000) ha, (v) Golen Gol (40800) ha, (vi) Madak Lasht (14500) ha, and (vii) Manur (6200) ha. A vantage point method was used for the estimation and the current population of Markhor in all the potential sites. The total recorded population of Markhor (male, female and fawn) in Chitral is 4582. The current research report confirm reasonable population of Markhor in Chitral compared to the available data in the literature. Our data confirm the effort made by the Khyber Pakhtunkhwa Wildlife Department for conservation of Markhor due to better management and improved wildlife protection. The positive role of community is also worth mentioning because not only the illegal hunting is locally refrained but external attempts are counted effectively. The population is increasing in Chitral and this will help in maintaining the trend of globally important species. Such protected areas can be effectively developed for other endangered species also.

BIRDS DIVERSITY OF KANHATTI GARDEN OF DISTRICT KHUSHAB

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Present study was conducted to assess the birds diversity and distribution of Kanhatti garden located in Soon valley of district Khushab. The duration of study was 6 months (February 2016 to July 2016). Total number of 64 species were observed from Kanhatti garden during six month study duration. However, total number of individuals recorded from Kanhatti garden were 4020. Maximum number of species observed from Kanhatti garden were 55 in the month of March while lowest number of species were recorded in month of July 45.

COMPARISON OF INTERNAL EGG QUALITY PARAMETERS OF *PAVO CRISTATUS* AND *PAVO MUTICUS*

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The study was conducted to compare the internal egg quality parameters of two common species of peafowl known as *Pavo cristatus* and *Pavo muticus* kept in captivity in Pak Peacock farm Bhalwal, district Sargodha, Punjab, Pakistan. The eggs (n=40) were collected, classified and weighed. Albumen diameter, Albumen height, Albumen

index, Albumen pH, Albumen ratio and Albumen weight, Yolk diameter, Yolk height, Yolk index, Yolk pH, Yolk ratio and Yolk weight, Shell thickness, Shell membrane thickness, Shell weight, Shell ratio, Haugh unit varied significantly ($P < 0.05$) between all the two species. The mean value of albumen index was ($6.60 \pm 0.04\%$) in *Pavo cristatus* and ($7.01 \pm 0.15\%$) in *Pavo muticus* while albumen ratio in *Pavo cristatus* was measured as ($49.83 \pm 0.13\%$) and in *Pavo muticus* was ($33.35 \pm 0.68\%$). Yolk index ($35.75 \pm 0.13\%$) in *Pavo cristatus* and ($20.22 \pm 0.96\%$) in *Pavo muticus*. Yolk ratio in *Pavo cristatus* was measured as ($33.52 \pm 0.57\%$) and in *Pavo muticus* was ($48.57 \pm 0.33\%$). Shell ratio in *Pavo cristatus* was observed as ($14.51 \pm 41.10\%$) and in *Pavo muticus* was ($11.74 \pm 0.58\%$). Haugh unit in *Pavo cristatus* was recorded as (75.45 ± 0.30) and in *Pavo muticus* was (73.48 ± 1.08).

COMPARISON OF EXTERNAL EGG QUALITY PARAMETERS OF *PAVO CRISTATUS* AND *PAVO MUTICUS*

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The study was conducted to compare the external egg quality parameters of two common species of peafowl known as *Pavo cristatus* and *Pavo muticus* kept in captivity in Pak Peacock farm Bhalwal, district Sargodha, Punjab, Pakistan. The eggs ($n=40$) were collected and weighed. Egg weight, egg length, egg breadth, egg volume, egg shape index and egg surface area varied significantly ($P < 0.05$) between two species. Higher egg weight ($94.44 \pm 0.42\text{g}$) was recorded in *Pavo cristatus* while lower ($85.44 \pm 0.59\text{g}$) in *Pavo muticus*. The value recorded for egg length was ($6.98 \pm 0.02\text{cm}$) in *Pavo cristatus* and ($6.32 \pm 0.02\text{cm}$) in *Pavo muticus*. Egg breadth size in *Pavo cristatus* was measured as ($24.55 \pm 0.12\text{ cm}^2$) and in *Pavo muticus* was ($24.94 \pm 0.10\text{ cm}^2$). Egg volume of *Pavo cristatus* was measured as ($85.02 \pm 0.44\text{ cm}^3$) and in *Pavo muticus* was ($78.22 \pm 0.57\text{ cm}^3$). Egg shape index in *Pavo cristatus* was observed as ($71.01 \pm 0.31\%$) and in *Pavo muticus* was ($79.08 \pm 0.11\%$).

BIODIVERSITY AND CONSERVATION OF WILDLIFE IN SAKESAR RESERVE FOREST PUNJAB SALT RANGE SOON VALLEY

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Sakesar reserve forest consist of different reserve forest including Rakh Sakesar Shahpur, Mianwali, Attock and Chitta reserve forest spread over an area of 7024 Acres. Sakesar reserve forests are located at an elevation of 3500 feet to 5010 feet the highest elevation in salt range. Sakesar reserve was declared as protected forest in 1896 with no grazing rights and is one of the best protected forest in the salt range. Climate of the area is harsh with minimum temperature up to -2C^0 in the month of January, while mean maximum temperature of 42C^0 in the month of June, Rainfall pattern of the area is scanty and mean annual rainfall is 600mm. Present study was conducted to establish base line data on wildlife and its habitat, threats to the biodiversity of the forest and proposed measures for the conservation of the biodiversity. Appropriate scientific methods were used to observe and record floral and faunal diversity of the Sakesar range forest. During the study 9 mammals, 45 birds, 7 reptiles and 7 amphibian species were recorded from the reserve forest. Major wildlife species of the area include Punjab Urial, Indian Wolf, Black Partridges, Chukor, Indian Pangolin and Grey Partridges. A total number of 60 plant species are identified in the study area among which dominant species included *Olea ferruginea*, *Acacia modesta*, *Zizphus nummalaria*, *Justicia adhatoda*, *Dodonea vicsosa* and different grasses. Major threats to biodiversity in area include forest fires, tree cutting, mining, illegal hunting, heavy grazing of livestock, drought, grass cutting. There is a need to protect flora and fauna from further degradation. Sustainable use of the resources may be promoted in the area through awareness raising and the entire Sakesar forest area network be declared a wildlife sanctuary for better conservation of flora and fauna.

A REVIEW OF BIRD CONTROL METHODS AT KARACHI AIRPORT

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This project examines the effectiveness of bird control method at Karachi Airport. Qualitative research methodology has been used, which contains auditory dispersing devices, visual dispersing methods, chemical repellent, other methods as independent variables and bird control methods as dependent variables. Using secondary data from CAA records of bird hits from 2011 to 2017 represent in graphical form. The primary data was collected through semi – structured interview from CAA concern authorities. The personnel involves in the sample belong to airside management, who are directly responsible to repel and disseminate birds from runway. The result reveals that Airport operators use bird shooting, crackers from auditory methods and from visual methods using scarecrow to repel birds from the vicinity of aerodrome. Although these methods are old but has proven the more efficient at Karachi Airport. The main recommendation for Pakistan CAA which is also mention in Doc 9137 AN/898 Part 3 to Conduct official study for determining the efficiency of bird control methods and make wildlife/Habitat management at Karachi Airport to counter future possible wildlife legal issues. This project directs more areas for further research on environment and wildlife perspectives. It will also be contribute in those field as well as aviation industry.

A PRELIMINARY SURVEY OF DIVERSITY OF DUCKS (GENUS ANAS) INHABITING SINDH PAKISTAN

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A survey was carried out to record the diversity of genus *Anas* that is a taxonomic group of dabbling ducks including the pintails, most teals, and the mallards. Survey were conducted in different subdivisions of Sindh including District Jamshoro (Kotri, Jamshoro, Sehwan, Thana Bulla Khan, Manjhand); District Naushahro feroze (Moro, Naushahro Feroze, Bhiria, Kandiaro, Mehrabpur); District Ghotki (MirpurMathelo, Daharki, Ghotki, Ubauro, Khangarh); District Hyderabad (Hyderabad City, Hyderabad rural, Latifabad, Qasimabad); District Mirpur Khas (Digri, Kot Ghulam Muhammad, Mirpurkhas, Jhuddo, Sindhri, Hussain bux mari, Shujabad); District Sanghar (Jam Nawaz Ali, Khipro, Sanghar, Shahdadpur, Sinjhor, Tando Adam Khan) from August to November 2018. Present exploration examined morphological parameters (body weight, body length, limbs length, plumage coloration, presence/absence of neck ringe, eye ring, iris coloration, bill coloration, bill shape) of ducks using scientific equipment and identified them using identification key and relevant taxonomic literature. The ducks found from Sindh province included three breeds of *Anus platyrhynchos* such as Magpie Duck (*Anus platyrhynchos domesticus*), Rouen Duck (*Anus platyrhynchos domesticus*) and Ancona Duck (*Anus platyrhynchos domesticus*), while Bernier's Teal or Madagascar Teal (*Anas bernieri*) was also observed to exist in the study area. Therefore, genus *Anas* of Sindh province was observed to consist of total four diversities viz: Magpie Duck, Rouen Duck, Ancona Duck and Bernier's Teal.

DIVERSTIY, DISTRIBUTION AND THREATS TO THE AVIAN FAUNA OF DIR (LOWER) KHYBER PAKHTUNKHWA, PAKISTAN

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This presence study was carried out from December 2016 to December 2017 regarding the diversity, distribution and threats to the avian fauna in different tehsils (Timergara, Samarbagh, Balambat, Lal Qila, Khal,

Munda and Adenzai) of Lower Dir Khyber Pakhtunkhwa, Pakistan. Data was collected by line transect method, point count method and direct method. In this study, the presence and observation of various species of birds was determined through their calls, direct sighting, and interrogating local forest staff, wild life department, villagers and with the help of designed questionnaire. The time schedule selected for field survey was early morning and before evening. A total of 132 bird species belonged to 14 orders and 44 families were recorded. The descending pattern of species richness was observed as; Timergara (n = 72), Samarbagh (n = 64), Munda (n = 63), Balambat (n = 55), Lal Qila (n = 52) Adenzai (n = 48) and Khal (n = 47). The highest species richness was recorded for Timergara (n = 72), whereas the lowest species richness was recorded for Khal (n = 47). The major threats to the survival of bird species recorded were increase in human population, plantation of exotic plant like Eucalyptus local name (laachee) is creating problems for some species of bird's especially *Alectoris chukar*. Deforestation of trees was the evidence of shelter damage and diversity, usage of herbicides and pesticides, habitat degradation and illegal hunting were the essential variables for the functioning and stability of an ecosystem, therefore, there is an urgent and dire need to shield avian diversity of district Lower Dir in order to protect the natural habitat of the area.

DIVERSITY AND MONITORING OF BIRDS IN UNDISTURBED FOREST AND ALONG HIKING TRAILS OF MARGALLA HILLS NATIONAL PARK

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We compared avifauna (spring-summer) of Margalla Hills National Park, Islamabad Capital Territory, among hiking trails (Trail 4, Trail 5 and Trail 6), undisturbed forest (undisturbed forest near trail 6 and undisturbed forest near trail 5) and human habitation (village Gokina, Talhar and Saidpur), and prepared audiospectrograms of some notable bird species. Three types of varied habitats were selected within study site. As many as 6660 individuals of 71 bird species were recorded from the habitats. The observed species diversity was highest at hiking trails with 56 recorded species (2798 individuals) with encounter rate of 27.98 birds per 15 minutes of the observation. The undisturbed forest featured the second highest species rich habitat with 44 species (1791 individuals) while village and human habitations had lowest bird diversity (33 species, 2071 individuals). The bird encounter rate was higher in the morning (40.38 birds per 15 minutes) compared to the evening time (26.22 birds per 15 minutes). The bird detection using traditional line transect resulted in the documentation of 71 bird species as compared to 56 species recorded through bioacoustics. The result highlights significance of intact and undisturbed forest area for the avifauna of the park.

POPULATION ASSESSMENT AND MANAGEMENT OPTIONS FOR HOUSE CROW (*CORVUS SPLENDENS*) IN URBAN LAHORE, PUNJAB, PAKISTAN

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House Crow (*Corvus splendens*) belong to family Corvidae comprising a large population in Pakistan, India, Nepal, Sri Lanka and all of Europe. House crow is omnivores in nature and mostly adapted with human surrounding. Present study was conducted from October 2017 to April 2018 to assess the population status and management options for House crow in Urban Lahore. Two study sites were selected which included Lahore Canal road from Mustafabad Bridge to Thokar Niaz Baig and Mehmood Booti (Lakhodair) landfill site. Population assessment was done during breeding and non-breeding season. Line transect and point count methods were used at Canal road and Mehmood Booti (Lakhodair) site, respectively. Population was estimated by using software DISTANCE® (version 6.0). Results revealed that the population density of House Crow at Canal road during non-breeding season was 27.42 individuals km⁻² (95% CI: 25.79 and 29.14) while during breeding season the population density was 18.806 km⁻²

(95% CI: 17.37 and 20.35). However at Mehmood Booti site population was only sighted during breeding season with a density of 15.3 individuals ha⁻¹ (95% CI: 4.55 and 5.79). Study revealed that the population of House crow was abundant due to availability of surplus food and shelter through garbage containers which were not properly covered. Sadqa food items thrown by citizens was another reason for their abundant population. Some crows were found dead at study site due to striking with vehicles and electric poles.

POPULATION ASSESSMENT AND MANAGEMENT OPTIONS FOR BLACK KITE (*MILVUS MIGRANS*) IN URBAN LAHORE, PUNJAB, PAKISTAN

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Black kites (*Milvus migrans*) belong to family Accipitridae with slender legs and long wings. They spend most of the time soaring, feeding on carrion along with various amount of live prey and are considered the most common and successful birds of prey in Pakistan. The present study was conducted from October 2017 to April 2018 to assess population status of Black kites and suggest measures/management options to manage their population in urban Lahore, Punjab province of Pakistan. For data collection, two study sites i.e. Lahore Canal from Mustafa Abad Bridge to Thokar Niaz Baig and Mehmood Booti (Lakhodair) landfill sites were selected. Surveys of study sites were conducted both in breeding and non-breeding seasons. Line transects method was used to count Black kites at Lahore Canal study site and Point count method was used at Mehmood Booti dumping site to count the kites in the area. All the kites roosting and flying were counted during surveys. The population estimation was calculated by using software DISTANCE® (version 6.0). Population density of Black kite at the Canal road was 13.72 individuals/ km² (95% CI: 12.29 and 15.32) in the non-breeding season while population in breeding season was 15.79 individuals/ km² (95% CI: 14.91 and 16.73). However, population density at Mehmood Booti was 3.47 ha⁻¹ (95% CI: 3.14 and 3.85) in non-breeding season while in breeding season population density was 5.89 ha⁻¹ (95% CI: 5.39 and 6.44). The incidences of kite attacks at Canal road area were recorded and information was also collected from local people through a questionnaire based survey. The study revealed that availability of food in the form of meat thrown by people in the name of Sadqa, cutting of trees along the Canal road, excess of garbage and illegal burning of garbage in the city provide great opportunity to Black kites for foraging and nesting and have made it as a dominant bird species over other native birds in Lahore city. The practice of sadqa meat has also changed the behaviour of Black kites and they often attack on passengers at canal road in Lahore.

HABITAT CHARACTERISTICS AND POPULATION DENSITY OF MUSK DEER (*MOSCHUS CHRYSOGASTER*) IN ASTROE VALLEY, GILGIT-BALTISTAN

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Musk deer (*Moschus chrysogaster*) belongs to the family Moschidae and genus *Moschus*. Musk deer are small solitary forest ruminants that inhabit the forested and alpine scrub habitats of mountains in Asia. Out of seven species of Musk deer only Himalayan Musk deer is found in Pakistan. It is now distributed in patches of its previous continuous range in the Himalayas. In Pakistan it is critically endangered. Musk deer population has experienced strong depletion, attributed to the loss of habitat, poaching and fragmentation. The present study was conducted to determine habitat characteristics and population density of Musk deer in Astore valley Gilgit-Baltistan. Line transect method was used for estimating its population. The results showed that the Population density of Musk deer is 1.01 animals/km² in the study area, occurring between 2938 m to 3492 m elevation. Quadrature method was used for habitat analysis of Musk deer in the study area. A total of 28 plants were identified in its habitat, of which 6 were trees, 6 shrubs and 16 were herbs. The dominant trees of the area were *Betula utilis*, *Pinus wallichiana*, *picea smithiana*, *juniperus spp.*, *Viburnum grandiflorum*, *Rosa maschata*, *Poa annua*, *Viola spp.*, *Polygonum amplaxicaul* and

Podophylum emodi. The preferred habitat of Musk deer was conifer forest with small ridges. Population of Musk deer is declining in the study area due to hunting and habitat degradation by humans and livestock activities. These factors should be addressed to conserve this endangered species in the study area. Results of the study will help in the conservation of this species and its habitat.

SURVEYS OF ZOOLOGICAL GARDENS IN PUNJAB TO ACCESS THEIR ROLE IN PUBLIC EDUCATION

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Keeping of wildlife in captivity was a continuous evolutionary practice for amusement of mankind. But now these captive sites have to play their role for public education and animal welfare as well other than recreation. According to World Association of Zoos and Aquarium (WAZA) annually more than 700 million visitors come to zoos of the world therefore these captive facilities are unique platform to educate the general public about animal welfare and conservation. The current research was intended to evaluate the role of Bahawalpur Zoological Garden, Marghazar Zoological Garden and Lahore Zoological Gardens for public education and animal welfare. To generate the data frequent visits of sites and meeting with zoo personals were arranged and a total of 500 questionnaires were also distributed among visitors from different walks of life. Results indicated that Lahore Zoological Garden was better among all the selected Zoos for education of masses and animal welfare. The findings also revealed that management of other zoos followed Lahore Zoo as role model in different aspects of animal handling like animal feeding charts, habitat designing and veterinary facilities. Even though Lahore Zoo still need improvement for animal record keeping, inbreeding issues, habitat designing, animal enrichment and collection plan, veterinary care facilities and nutrition. Among zoos under surveys a public education and awareness programme was only present at Lahore Zoo although it was not properly planned and designed to convey the message of animal welfare in true sense but it was still source of attraction and motivation for 60% of visitors especially students.

SURVEILLANCE OF DISEASES IN WILD MAMMALS OF ZOO SAFARI LAHORE FOR THEIR BETTER CARE AND CONSERVATION

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In 20th century captive and semi-captive housing conditions are emerged as an important tool for conservation of wildlife. But these facilities are facing many challenges like infectious diseases and shocks for upkeep of wildlife. The study was planned to access the causes of death in wild mammals and to provide data regarding prevailing diseases for better care and conservation. Data regarding animal care, diseases and postmortem findings was collected by frequent visit of the site from 2016 to 2017. A total of 92 deaths of 11 mammal's species were recorded at site. Among them carnivores were *Panthera tigris* (endangered) and *Panthera leo* (vulnerable) while herbivores were *Gazelle benettii*, *Antelope cervicapra* (endangered), *Dama*

dama, *Axis axis*, Muntjacs deer, *Hylaphus porcinus*, *Equus quagga*, *Ovis orientalis* (vulnerable), *Boselaphus tragocamelus*. In total 83.6% deaths were of herbivores while carnivores' death toll was 16.3% in two consecutive years. The herbivores' mortality was 81.6% and 76% in 2016 and 2017 respectively. In 2016 carnivores' mortality was 18.1% and 13.5% in 2017. Gender wise mortality records showed that males' deaths were 57% while females were 42.8%. Age wise death records indicated that 86% was cubs while 13% was adult in carnivores. In herbivores fawns' death was 10.3% and 89% was for adults. Early age mortality was significantly high in carnivores. Highest 50.9% mortality was recorded in *Antelope cervicapra* during 2016 due to Malignant Catarrhal Fever. In 2017 highest 24.7% & 24.9% mortality rates were recorded in *Axis axis* and *Hylaphus porcinus* respectively due to hemorrhagic enteritis, traumatic injury, jackal and stray dogs' attacks, respiratory distress and cardiac arrest. In carnivores causes of death were septicemia, anemia, gastroenteritis and heart diseases. Overall infectious diseases (viral/bacterial) were found to be major cause of deaths while shocks/stress were second in this regard. But lack of field staff, under developed veterinary facilities, improper record keeping, lack of animal's behavior information and lab tests facilities increases the problem many folds. It is concluded that better management and care is more suitable than treatment.

ABUNDANCE AND HABITAT ANALYSIS OF GREY FRANCOLIN (*FRANCOLINUS PONDICERIANUS*) IN TAUNSA WILDLIFE SANCTUARY

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Grey Francolin (*Francolinus pondicerianus*) belongs to order Galliformes and included in family Phasianidae. It is an important game bird of Pakistan. Present study was conducted to estimate its population density and to determine its habitat association in Taunsa Wildlife Sanctuary, Punjab from February 2017 to July 2018. The study area was divided into two different habitat types i.e., i) cultivated land ii) forest and associated grass land for collecting data. In each habitat type, three fixed transects having length of 200 m and width of 50 m, were selected and marked. Data for population density was collected along these fixed transects in early morning before sunrise and afternoon before sunset. For habitat analysis, vegetative survey of selected study sites was conducted by using quadrature method. Ten quadrates were taken on each fixed transect. Relative density, Relative Frequency, Relative Cover and Importance Value Index for all plant species were recorded from habitat of Grey Francolin. Physical features within selected habitat were also noted such as vegetation, elevation, and water availability. Population density of Grey Francolin in the study area was estimated as 1.03 birds / ha. Population densities in cultivated land, and natural forest were 1.29 birds/ha and 0.78 birds/ha, respectively. Thirty-one plant species were recorded from the habitat of Grey Francolin, out of which 11 were trees, 6 were shrubs, 6 were herbs and 8 were grasses. Major tree species were, *Acacia nilotica*, *Albizia lebbbeck*, and *Dalbergia sissoo*. Dominant shrub species included *Azadirachta indica*, *Withania somnifera* and *Datura innoxia*. Whereas the dominant herbs species were *Achyranthus aspera*, *Chenopodium album*, and *Oxystelma esculentum*. The main grasses included *Cynodon dactylon*, *Desmostachya bipinnata* and *Boerhavia procumbens*. Most preferred habitat of Grey Francolin in the study area was cultivated land having population density of 1.29 birds/ha. Important plant species of this habitat were *Acacia nilotica* (IVI= 57.59), *Tamarix dioica* (IVI=36.03), *Dalbergia sissoo* (IVI= 29.78), *Albizia lebbbeck* (IVI= 22.12). *Cynodon dactylon* (IVI= 9.18), *Desmostachya bipinnata* (IVI= 8.85), and *Saccharum arandinaceum* (IVI= 6.16). Forest and associated grass land habitat was dominated by plant species of *Acacia nilotica* (IVI= 44.32), *Albizia lebbbeck* (IVI=38.37), *Dalbergia sissoo* (IVI= 21.97), *Eucalyptus camadulensis* (IVI= 17.94). *Withania somnifera* (IVI= 21.91), *Cynodon dactylon* (IVI= 10.68), and *Desmostachya bipinnata* (IVI= 9.60). The most important features of cultivated land were crops, and trees of *Acacia nilotica*, *Albizia lebbbeck*, *Dalbergia sissoo* which provide cover to the grey francolin.

DISTRIBUTION OF ORDER CUCULIFORM IN DISTRICT BADIN, SINDH, PAKISTAN**Misbah Arain, Kalsoom Shaikh, Iqra Arain, Ghulam Sarwar Gachal***Department of Zoology, University of Sindh, Jamshoro 76080*

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Cuckoo birds have wide distribution, but their taxonomic and ecological status in District Badin (6,726 km²), Sindh, had remained unexplored previously, therefore, present study was carried out to record their taxonomic status in district Badin from March, 2017 to October, 2018. The cuckoos were identified using identification key and related scientific literature. The results of present study showed existence of one species of cuckoos "*Eudynamys scolopaceus*" belonging to order Cuculiformes under family "Cuculidae". Morphological characteristics of males were observed as colour of body was bluish black, bill pale or greenish grey, plumage black, iris bright red, feet and legs grey, under part of body feathers were observed as white, claws straight and large sized, tail long and bill down curved. However females were observed to possess following characteristics: colour of body brownish on the crown and rufous streaks on the head, back rump were observed dark brown with white spots, wing coverts dark brown with white spots, underparts whitish and heavily striped, light brown lines on forehead, nostril was observed dark coloured, colour of bill pale greenish grey, while colour of legs and feet was observed grey. It was thoroughly recorded that the study area "District Badin" embraced only one species "*Eudynamys scolopaceus*" widely distributed in all kinds of habitats including agricultural, urban and sub urban areas.

DISTRIBUTION OF *STREPTOPELIA DECAOCTO* (EURASIAN COLLARED DOVE) IN SINDH, PAKISTAN**Iqra Arain*, Kalsoom Shaikh, Misbah Arain and Ghulam Sarwar Gachal***Department of Zoology, University of Sindh, Jamshoro 76080, Pakistan*

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Streptopelia decaocto (Eurasian collared dove) expands so rapidly, competes with other birds species efficiently, feeds heavily on variety of crops (hence called crop pest), and carries circovirus which causes illness and mortality in the pigeons, thus its existence sometimes results in negative impact on balanced ecosystem. In this context, present study was proposed to explore Sindh province (140,914 km²) to record morphology and existence of *S. decaocto*. This study also focused on localization of nests and observation of fledglings of *S. decaocto* to confirm rate of their distribution in different types of habitats including cropland, scrubland, suburban and urban areas. Field surveys were carried out randomly from January to August 2018. Distribution of Eurasian collared dove was observed in all 14 study areas at extensive level, though existence of the species in question was not found merely in Districts of Shahed Benazirabad and Sukkur. The habitats from where *S. decaocto* was found recurrently and profusely was cropland, however availability of species was observed very rare in urban and suburban habitats.

SPECIES COMPOSITION, RELATIVE ABUNDANCE, DIVERSITY AND DISTRIBUTION OF PHEASANTS IN KHYBER PAKHTOONKHTA PAKISTAN**Rida Adam Khan¹, Lubna Shireen² Nehafta Bibi^{1*} and Munawar Saleem Ahmad³**¹*Government Girls Degree College # 1 Mansehra*²*Department of Zoology Hazara University Mansehra*³*Department of Zoology, University of Swabi*

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Mansehra is a city located in Mansehra district in the eastern part of Khyber Pakhtoonkhwa Province of Pakistan with total area 1,340 km (520 sq mi) and elevation, 1,088 m (3570 ft). The climate is much cold in winter and pleasantly warm in summer. Dhodial, Jaborri Upper Siran and Icherrian were the study sites for survey. The study was carried out to determine the composition and Diversity of Pheasants species, residential and migratory pheasants,

and factors responsible for their habitat loss. Direct as well as indirect methods were used for data collection by using binoculars, DSLR and interviews were conducted from wildlife staff, residents and hunters. Among Pheasant species, Monal (*Lophophorus impeyanus*), Khalij (*Lophura leucomelana*), Koklass (*Pucrasia macrolopha*), and Trygopan (*Tragopan melanocephalus*) were present (observed in early morning and evening). Among them Khalij (*Lophura leucomelana*) is in great number while Monal (*Lophophorus impeyanus*) and Koklass (*Pucrasia macrolopha*) count were few. Hunting is the main cause of their habitat disturbance which lead to the extinction of Cheer (*Catopus wallichi*) where Trygopan (*Tragopan melanocephalus*) and Monal (*Lophophorus impeyanus*) are on extinction risk. (*Tragopan melanocephalus*) is now only found in Palass valley Kohistan. Finally the data will provide strong arguments and strategy for the preservation and conservation of Pheasants by keeping them in captivity and proper check and balance over hunting.

IMPACT OF HUMAN SETTLEMENTS ON DECLINING NUMBER OF WATER FOWLS IN MANSEHRA DISTRICT KPK

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Urbanization and its associated effects cause dramatic environmental changes at multiple scale including shifts in land-cover, and introduction of exotic species. Wetland is an important breeding and wintering area for water fowls. Losses of wetlands directly lead to losses in habitat and fragmentation of water fowls habitat. This study was carried out on Riverine habitats, low wetlands with the objective to identify and prioritized foremost factors influencing on waterfowls population including environmental factors. Surveys were conducted on proposed sites, showed that geese and duck species found in this belt, including Mallard (*Anas platyrhynchos*), Red headed duck (*Aythya Americana*), Pin Tails duck (*Anas acuta*), Garne Teal (*Anas crecca*), Shoveler (*Spatula clypeata*), Pochard (*Aythya ferina*) are migratory seasonal water birds. All duck varieties are game bird's getting declined by hunting. Seagul and Snipe are local waterbirds and not influenced by hunting. Climatic conditions are also responsible for decreased population of waterfowls. Rudy shell duck specie is decreased due to influence of climate. Migration of seasonal aquatic bird crane is also getting decreased in these areas. The contributed work emphasizes the need to develop adaptive management plans for waterfowls population. This endeavour should take into account climate forcing on waterfowls population, the apparent impact of other species, as well as possible interaction between these two.

PREVALENCE OF ILLEGAL TRADE OF WILDLIFE IN PAKISTAN ON SOCIAL MEDIA

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The present study was designed to evaluate the prevalence of illegal wildlife trade in Pakistan. The study was conducted for a duration of five months from August 2016 and ended in December 2016 from different cities of Pakistan using social media. During the study period, activities of 65 groups and 89 pages on various social media sites was collected by keeping records of posts selling wildlife. It was observed that popular species traded included variety of birds such as African grey parrot, Alexandrine parrot, Rose ringed parakeet and different species of Macaws. A total of six percent endangered animal species were found in the trade both on facebook pages and ad groups, whereas 12 % near threatened and 21 % vulnerable species were found. About 54% of animal species were native and were included in one of the three CITES appendices, showing that a CITES permit are required for their trade. About 51% non-native species were being traded internationally. This illegal online trade is a growing business

in Pakistan. Furthermore, the overall analysis revealed that the trade in animals online is increasing as more and more people are becoming aware of the ease of earning money via this method. It also showed the rising demand for domesticated wild animals both native and exotic as pets in Pakistan. If this trend continues, it will put pressure on some endangered and vulnerable species. There is dire need to regulate the online trade of vulnerable and endangered species on social media.

BIRD SONGS, NATURE'S MELODIOUS VOICES

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Bird songs have been fascinating humans since the very beginning of time. Defining the vocal repertoire provides a basis for understanding the role of acoustic signals in sexual and social interactions of an animal. Birds use their songs mainly to attract mates and to threaten conspecifics. There are recognizable local dialects within each species. Bird songs develop in two phases, the sensory and sensorimotor phases. Sensory phase develops several days after hatching, while sensorimotor phase begins when the juvenile males begin to twitter. Sensitive phase is very critical. Several bird species like zebra finches and white-crowned sparrows are age-limited learners, while others, like male canaries, are open-ended learners. Once songs are crystallized, songbirds are less dependent on hearing for normal song production. As regards, neural circuits, in canary, descending motor pathway mediates song production, while anterior forebrain pathway mediates song learning. Spectrogram analyses have demonstrated that birds produce two kinds of vocalizations, a) typically short and simple calls, and b) usually lengthy and well-structured organized songs mainly associated with reproduction and aggression. Birds of the cuckoo family (Cuculidae) are although famous for loud vocalizations but they are non-vocal learners. Our laboratory is now engaged in deciphering further the songs of Asian Koel. Previous work demonstrated "cooee call", "wurroo call" and "whik call" produced by the Asian Koel. My laboratory has identified four other call types that we categorized as; cooee", "type 1 coegh", "type 2 coegh" and "coe", for the male Asian Koel and, also measured various structural and temporal parameters for all call types. Presently, we are working on the individuality of each call type and have come up with interesting findings. Our findings have enriched the biological knowledge about male Asian Koel's vocalizations and provide a foundation for future comparative studies among different species of other cuckoos. Future studies should focus on exploring the entire vocal repertoire of male Asian Koel and to understand the functions of the vocalizations in sexual selection, social interaction and individual recognition.

5. BIODIVERSITY

SEASONAL VARIATION IN ENVIRONMENTAL VARIABLES AND THEIR IMPACT ON MOLLUSCAN FAUNA IN NARMADA RIVER INDIA

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Molluscs comprise an important group of aqua fauna by way of their contribution to ecosystem stability. The river Narmada is the third holy and fifth largest west flowing river of India and the biggest west flowing river of the state M. P. India. The present study was carried out for the period of 12 months from March 2015 to February 2017. Two sampling sites were selected namely the Omkareshwar and Mandleshwar for studying the ecological distribution of molluscans. In the present study average population size recorded during summer season was 44.32, during monsoon season 17.17, post monsoon season 17.04 and during winter season 31.96. The value of Shannon diversity index (H) recorded was low during monsoon season (2.85) at Omkareshwar and gradually increased during post monsoon season (3.07), however dominance (D) index was lower in monsoon and higher during post monsoon and winter seasons. The value of Correlation coefficient (r) indicates that there was positive correlation between the molluscan population and environmental variables like temperature, pH and biological oxygen demand. Our study raise an important point concerning the regular monitoring of physico-chemical as well as biological parameters of the Narmada river which will be useful in maintaining the productivity of the river. This study shows that the diversity of molluscan fauna alters with the change in environmental variables.

BIODIVERSITY OF INVERTEBRATE IN SOME SELECTED AREAS OF DISTRICT BAGH (AZAD JAMMU AND KASHMIR) PAKISTAN

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The present study was designed to find species composition, diversity of invertebrates existing at different trophic level of food chain in coniferous forest of District Bagh. The study was conducted from September 2017 to September 2018. We recorded 61 species of insects belonging to 51 families. Among the identified species of invertebrates 32 species are herbivore, 19 species are carnivore (insect predator) and 8 species of omnivores and 2 species are scavengers. The herbivore insect species feed on 134 plants of the study area. The greater population density of herbivore, indicate that plants are under more threat to insect pest. The present study provide base line information about the insect community of forest and need further screening of insect pest species to control pest out break for conservation of coniferous forest District Bagh.

MORPHOMETRIC VARIATION OF SPINEY TAILED LIZARD (*SAARA HARDWICKII*) FROM CHOLISTAN DESERT, PUNJAB, PAKISTAN

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This study has provided the data on morphometric of spiny tailed lizard (*Saara hardwickii*) in the area of lesser Cholistan desert, Punjab, Pakistan. Adult spiny tailed lizards were collected from poachers with the help of

Punjab wildlife department, Pakistan. In Cholistan, these lizards are often illegally collected and sold in various parts of the country for their use in medicine and for making oil from its fat, for which poachers claim that; it is effective in joint pain relief and provides strength to the male sex organ. They were captured in the season of early winter. Among them 9 were male and 8 were female. Total body mass (g) were recorded (Female=142-182g to Male=200-371g). Total body length (BL) (range in male 160-200mm and female was 120-170mm) was also measured. Snout vent length (SVL) was measured for all spiny tailed lizards (range 120-150mm in male and 110-130mm in female). Tail length (TL) was noted (range 130-190mm in male and 140-160mm in female) and spines mark on the tail were also counted which were 25-31 in male and 21-25 in female. All measure was made by using digital LCD vernier caliper and weighing balance (SK-5K Model) having range of 1g to 5kg. Comparison between male and female morphometric was made using the Analysis of Variance (ANOVA). The *f*-ratio value is 21.4353. The *p*-value is .000327. The result is significant at $p < .05$. Detail study is required on ecology of spiny tailed lizard for its conservation in lesser Cholistan desert; one of main area of its distribution in Pakistan.

BIOSTATUS OF INDUS RIVER DOLPHIN (PLATANISTA MINOR) IN D.I. KHAN RANGE OF KHYBER PAKHTUNKHWA, PAKISTAN

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To evaluate the status, distribution and threats to the Indus River dolphin, *Platanista minor*, its range in KPK, was surveyed in March 2018. The study comprised of Indus River and its tributaries. The aggregate of best group estimates produced an abundance estimate of 43 dolphins. Around 51.2% (0.81 dolphin/km) of the dolphin populace happened in 27 km of river length from Miran onwards to Ramak in the Indus River, 39.5% (0.37 dolphin/km) of the dolphin populace happened in 46 km of river length from D.I. Khan Bridge to Miran in the Indus River and 9.3% (0.13 dolphin/km) of the dolphin populace happened in 30.5 km of river length from Saggu, close Meetapur town to D.I. Khan Bridge. The most noteworthy population of dolphins was among Miran and Ramak in the Indus River. An articulated increment in dolphin experience rate and plenitude was seen a downstream way. Dangers to dolphins incorporate excessively vessel movement, aggravation by the crane and duck seekers, large amounts of anthropogenic risk and no successful preservation. River dolphins are especially helpless against the exercises of people on account of their limited natural surroundings. Dangers differ topographically in their significance, but generally include coincidental murdering during fishing operations, territory misfortune and populace discontinuity from water development. Deliberate killing for dolphin items likewise undermines the creatures.

SEASONAL VARIATIONS IN DIVERSITY OF MACRO INVERTEBRATES OF CHASHMA LAKE MIANWALI, PUNJAB, PAKISTAN

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The aim of the study was to evaluate the macro invertebrates diversity at Chashma Lake from April 2016 to March 2017 on monthly basis. The Shannon diversity index, species richness and species evenness of macro invertebrates was calculated. The maximum diversity was recorded in month of August (2.91) and minimum diversity was observed in November (2.48). At Chashma lake the range of species richness of macro invertebrates was (15.72 to 24.76) and the range of species Evenness of macro invertebrates was (0.71 to 0.94). The maximum diversity was investigated in July (3.03) and minimum diversity was observed in November (1.95).

DIVERSITY OF ANURANS (FROGS AND TOADS) IN DISTRICT SWABI

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The present study was conducted to investigate the diversity of amphibians at selected areas of district Swabi, KPK which was the 1st study in this area. Frogs and toads were collected from indoor and outdoor sites (streets, fields, barren grounds, banks of water bodies). Identifications were undertaken by the help of Amphibians key of Pakistan by Muhammad Sharif Khan. Amphibians and reptiles are the most neglected and least studied wildlife groups in Pakistan. Pakistan is an amphibian poor country, because of existing dry sub tropical environmental conditions. We collected 157 sample having 4 species which are further classified into 3 genera belonging to 2 families. The species include *Bufo viridis zugmayeri*, *Bufo stomaticus*. from one family and *Fejervarya limnocharis*, *Hoplobatrachus tigerinus* to other. Toads are essentially nocturnal, but also become active in the daytime during breeding season.

AN ANALYTICAL STUDY OF WATER QUALITY OF HABITATS OF AMPHIBIAN FAUNA IN SINDH, PAKISTAN

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The present study was proposed to investigate the status of amphibian habitats into agricultural ponds of Sindh province. In this context, 26 aquatic habitats were selected for the regular water sampling in order to analyze their chemical quality through the parameters including electric conductivity (EC), total dissolved solids (TDS), total hardness (T. Hard), total alkalinity (T. Alk), sulphate (SO₄) and phosphate (PO₄). Analytical study of water quality revealed value of all parameters extremely high than permissible limit that may make quality of habitats unsuitable for the water breathing animals like amphibians. The value of parameters was analyzed as followed: EC (2506.3±1139.1), TDS (1712.1±581.2), T-Hard (534.2±170.5), T-Alk (284.4±65.9), SO₄ (451.8±122.1) and PO₄ (429.4±94.3). Water quality of habitats may have an adverse effect on population of amphibians as their spawning, hatching, metamorphosis and growth occurs in water where they remain confined until completion of their development into adults. Such water quality may deteriorate amphibian populations of study area at large and may induce variety of physiological and physical abnormalities into them. Therefore, conservation efforts may be taken urgently to save amphibians from their possible decline in future.

ASSESSMENT OF ANURAN SPECIES INHABITING DISTRICT NAUSHAHRO FEROZE, SINDH, PAKISTAN

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Order Anura is the only order of class amphibia that exists in Pakistan, however some areas of Sindh are yet to be explored for the confirmation of diversity of frogs and toads. In this context, present study was proposed to explore "Naushahro Feroze district" where no relevant investigation of anuran diversity was ever conducted before this study. Field surveys were carried out from January to August 2018 in different types of habitats such as scrubland, cropland and suburban areas where prevalence of amphibians was observed. Morphology of discovered amphibian species was thoroughly examined and identified as *Hoplobatrachus tigerinus* and *Euphlyctis*

cyanophlyctis. Morpho-taxonomic variation was utterly absent in members of both distinct species. Some minor variations in body coloration and pattern of patches were recorded. Existence of only two anuran species falling in two genera (*Hoplobatrachus* and *Euphlyctis*) indicated that the study area embraced extremely poor diversity of order Anura as compared to other areas of Pakistan. Arid climate, habitat destruction and anthropogenic encroachment may be the reasons that Naushahro feroze district fails to attract majority of anuran species, hence apt legislation is strongly recommended to encourage for conservation of amphibians.

NEW RECORD OF SEA ANEMONE *PSEUDACTINIA SP.* & *BUNODOSOMA SP.* (CNIDARIA: ANTHOZOA: ACTINIARIA) FROM THE ROCKY SHORES OF THE KARACHI COAST, PAKISTAN

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Sea anemones are marine invertebrate live throughout the world's oceans, from the poles to the equator as usually exist only in shallow waters. Sea anemones belong to Order Actiniaria, Subclass Zoantharia sessile with some swimming forms, having nematocysts. Sea anemone occupied a wide range of habitat, i.e. among fouling communities, coral reefs, and rocky shore especially in the intertidal zones. Sea anemones have a rich potential as a source of therapeutic agent. During the present study, sea anemones randomly collected at high, mid and low tide mark collected from intertidal rocky shores of Buleji and Manora coastal areas of Pakistan. Initially the collected sample preserved in alcohol and then were photographed for identification and recording of external morphological features and the internal element includes three criteria for identification. Actinopharynx, Siphonoglyph and Mesenteries. Two new species of Actiniaria (Sea anemones): *Pseudactinia flagellifera* and *Bunodosoma sp.* identified. The current study attempted to furnish the information about taxonomic identification and extent of distribution of the Sea anemone in coastal waters of Pakistan.

ASSESSMENT OF THE MAMMALIAN DIVERSITY IN MARGALLAH HILLS NATIONAL PARK, ISLAMABAD

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Exploring mammalian diversity is a prerequisite to understanding the health of an ecosystem and plays a pivotal role in conservation planning. For this purpose a study availing sign surveys and camera trapping was conducted in the Margalla Hills National Park Islamabad Pakistan. The sign surveys were carried out from November 2017 to February 2018 while camera trapping lasted from 18th of February to 11th of March 2018. This was the first study of its kind in 38 years since the area was notified as a national park. The study involved 30 motion-triggered infrared digital trail cameras deployed (each for 13 trap nights) across the study area in different potential sites making a total of 364 trap nights and covering 30 grids of 1 km² each with a minimum distance of 500 m between each camera station. The camera traps captured eighteen species of mammals representing thirteen families that included red fox, Indian porcupine, wild boar, masked civet, golden jackal, rhesus macaque, common leopard, leopard cat, barking deer, Indian civet and Indian hare. Jungle cat, mongoose, Indian pangolin, yellow-throated marten, hedgehog, Rat (genus *Rattus*) and Himalayan black rat each were captured at only one station. Highest photo-capture events (229) were recorded for red fox though wild boar generated the highest number of photos (3537). Mammalian species accounted for 28.91 % of total photo captures. Human, birds, livestock and domestic animals accounted for 42.80%, 5.64%, 13.54% and 4.11% respectively while 4.9% photos were falsely triggered. The Relative Abundance

Index (RAI) was the highest for Red Fox (36.64) moderate for Indian porcupine (17.12) wild boar (14.72) rhesus macaque (10.08) and masked civet (9.44). Whereas golden jackal, barking deer, Indian civet, common leopard, leopard cat, jungle cat, and yellow-throated marten had the lowest abundance. Species Richness was estimated using five estimators namely ICE, ACE, Chao1, Chao2, and Jack1. Chao2 gave the highest estimate of 38.3, ICE gave 28.32, Jack1 gave 24.77, ACE gave 19.09 and Chao1 gave an estimate of 18.5 approximately the same as the observed no of species). The current study recorded 18 mammalian species including some rare and threatened ones such as common leopard and Indian pangolin and other rare species like leopard cat and jungle cat which were not reported for very long from the area. In contrast to past instigations which were largely based on expert judgments anecdotes, animal spoors current study provides definitive detections of several iconic species. Though a shear amount of photo belonged to humans and livestock (55%) or non-target species (16%) photo capture of mammalian species (29%) was adequate to recognize species. Presence of large predators like common leopard without serious conflicts is a significant finding which could be correlated to the availability of natural prey (wild boar, barking deer) adaptable nature of the cat however seasonal surveys are recommended to better capture species diversity and movements in the area. The study reports great ecological potential of the area in the form of species richness as well as an immense challenge in the form of huge human and livestock movement in core areas of the park. The results shall inform the management strategy adopted by the park management so that human substance, recreational and ecological needs are balanced.

FIRST RECORDS OF SYMPHYLA, GASTROTRICHA, GNATHOSTUMILIDA AND ARACHNIDA FROM COASTAL ZONE, KARACHI

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While working on interstitial fauna several specimens of many uncommon phyla like Symphyla, Kinorhyncha, Archiannelida, Gastrotricha, Gnathostumilida along with the common Arthropoda were collected from coastal sand of Sand Spit, Karachi. Most people do not notice or recognize them, primarily due to the secretive nature and small size of these animals. Out of them Symphyla and Gastrotricha, Gnathostumilida are selected for this paper. In the mean while from the same intertidal region of Karachi specimens of spider of family Saltidae (Arachnida) from crevices and centipede of family Scolopendridae (Arachnida) after breaking rocks were collected. All of these groups are being reported from intertidal zone of coastal area for the first time. Each group/species is photographed illustrated and described.

IMPACT OF POLLUTED SEWAGE WASTEWATER ON DIVERSITY AND DENSITY OF SOIL MACRO-FAUNA AMONG CAULIFLOWER (*BRASSICA OLERACEA* L. VAR. *BOTRYTIS*) AND TOMATO (*SOLANUM LYCOPERSICUM* L.) FIELDS

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Soil communities are principal source to run the bio-geo-chemical cycling in soil and support to above-ground relevant cycling for the sustainability of ecosystem. Soil acts as shelter and source of nutrients for their growth. Keeping in view their importance in soil decomposition and substantial part of the global biodiversity, determination of their diversity, richness, evenness and density are required for ecological studies, habitat management and conservation programs, with regard to polluted and non-polluted water irrigation system. The objective of the study was to determine the impacts of water pollution on diversity and density of soil macro-fauna among tomato and cauliflower (control and treated) fields. The Diversity index was recorded maximum in tomato control field (2.937). The Dominance was high in tomato treated field (0.497). Evenness was higher in cauliflower treated fields (0.846).

In tomato fields higher richness was recorded in tomato control field (75). The t-test analysis showed significant results ($t = -0.98516$, $p < 0.05$) by comparing the means of control fields and treated fields. Average no. of specimens per m² in control fields (tomato & cauliflower) were higher (15.30) than in treated fields (tomato & cauliflower) (8.91). Analysis of variance among tomato and cauliflower (control and treated) showed, non-significant ($p > 0.5$) difference between average number of specimens. By present findings, it was referred that soil pollution by polluted water irrigation system, in any way is hazardous for soil macro-fauna.

MORPHO-MOLECULAR CHARACTERIZATION OF THE PHYSA (GASTROPODA: PHYSIDEA) USING MOLECULAR MARKERS

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Physids are members of the class gastropoda which belongs to Phylum Mollusca. They have very important position in food web and act as bio indicators, pests and intermediate host. Snail species belonging to genus physa was collected from different water bodies of Faisalabad and identified up to species on the basis of morphometry for further characterization on the basis of molecular markers. Morphological parameters were analyzed using linear regression analysis showing pronounced allometric growth exists in *P. acuta* followed by *P. fontinalis*, however in case of *P. gyrina*, the aperture length was not found a major contributing factor in shell growth. High level of genetic diversity was revealed by polymorphic RAPD and SSR markers. The multivariate analysis was conducted to generate a similarity matrix based on Nei's Unweighted group of Arithmetic Means Average (UPGMA) to estimate genetic distance and relatedness in snail genotype. The genetic similarity value ranged from 70 percent to 77 percent among the three genotypes of snail under study. Total of 12 polymorphic loci were found out of 38 RAPD markers. The present research findings will be helpful for future specie specific identification of snails species.

DIFFERENTIAL DIAGNOSTICS OF ALLOPEAS GRACILIS (GASTROPODA: SUBULINIDEA) USING TYPOLOGICAL SPECIES CONCEPT

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This is a taxonomic study for the characterization of the *Allopeas garacilis* (Gastropoda: Subulinidae) in Faisalabad, Punjab, Pakistan. The genus *Allopeas* is previously reported from various parts of the world i.e. , India, Iran and in Arabian countries, however this is its first report from Pakistan. The diagnostics are on the basis of conchological characters on the basis of typological species concept. Total 100 specimens were collected from different areas of Faisalabad, Punjab, Pakistan out of which 20 adult specimens were selected. The average shell length, shell width, aperture length and aperture width was 6.85, 2.34, 2.2 and 1.37 respectively. The regression equation shows insignificant allometric growth in *Allopeas gracilis* which makes it identifiable when compared with *Zootecus insularis* as these two species belong to the same family with same habitat.

ESTIMATION OF DIVERSITY AND RELATIVE ABUNDANCE OF BERSEEM CROP IN DISTRICT SIALKOT BY USING QUADRATE METHOD

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A field study related to biodiversity and relative abundance of insect species in berseem crop were conducted in district Sialkot. Berseem crop was selected as it is a fodder crop and provides protein and many essential mineral to

animals. And in Pakistan it is an important leguminous crop for livestock feeding. In order to collect insects pests and their natural enemy's Quadrates method were used. Quadrates were thrown randomly and then insects are collected from them. A total of 903 specimens were collected and preserved. Maximum specimens were observed during the month of April. *Paederus fuscipes* showed the maximum numbers (120) followed by *Spodoptera exigua* (84), *Spodoptera litura* (64) and *Chrysoschusco baltinus* (56). Among aphids *Rhopalosiphum maidis* were observed. Among the family Acridae, *Acrida exaltata* (23), *Mermiria bivittata* (20) and *Oxya hyla* (19) were observed abundantly. Some species of *Camponotus itoi* were also observed abundantly. The result indicated the presence of high diversity on berseem as it harbors the large numbers of pest and predators due to its soft and juicy leaves, tender stem, high leaf to stem ratio and fast regrowth.

SECTION – V I

GENERAL ZOOLOGY

CREATION IS NOT ONLY THE MOST PRIMITIVE BASE OF EVOLUTION BUT ALSO THE COMPULSORY NEED OF EVOLUTION

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The word science may be define as "the study and copy of nature and natural phenomenon to understand the secretes of universe". The word science may be taken from the word Hikma in Arabic, Danai in Urdu and Sense in English, which means the internal wisdom, observed by the help of sense organs or additional instrument or apparatus or machine. Science always speak about truth, fact, actualness and perfectness. Science believes in $2+3 = 5$ not in 4 or 6. The creation took place without pre-existing sample/example/species, where as the evolution could not proceed/takes place without pre-existing sample/example/species. Creation is more primitive than evolution, as the things/specimens/organisms/species were 1st created and then the evolution took place in them according to the need of the specimens/species or according to the requirement of the time and environment. The evolution takes place in gene, shape, size, color, height, function, need, ability, habitat and fitness according to the need of species or environment by the passage of time in living things, where as evolution also took place in non living things such as light, temperature, humidity, elements, minerals, gases, charges, ph, composition, concentration, phase, equilibrium etc. Comparative study method of literature from books, journals and internet was used, especially the study of evolution of living things including plants, animals and Humans. In case of plants the potato has no seeds then how its first plant came into existence? Ultimately created. Similarly the Hydra an animal of multicellular level reproduce through four methods, binary fission / bifurgation , budding/natural cloning and regeneration as asexually but how the 1st individual of hydra who came into existence? Ultimately it was created, as for sexual reproduction of hydra (4th method), two individuals are needed. Similarly 1st the egg of hen was came into existence or the pair of hen was created who laid the egg. Similarly incase of humans how the humans came into existence only by creation, because the first member of humans Aadam was created, then Eve evolved from the Aadam through NMP by budding/natural cloning to make the pair level of humans, and when the pair of humans was maintained then sexual reproduction took place which is continued till today. The eve evolved from the body of Aadam which is evolution. Evolution could not takes place without the help of creation. Both creation and evolution are facts, both should be considered. Neither the creation nor the evolution could be denied, because both support to each other. All existing races of *Homo sapiens* arose from a single ancestor Eve (Benton 2005) and Eve evolved from Aadam, reported online in the recent years (Tariq 2011, 2014).

SECTION – V I I

POSTER SESSION

SELECTIVITY IN THE EXPLOITATION OF FLORAL RESOURCES BY APHIDOPHAGOUS HOVERFLIES (SYRPHIDAE: SYRPHINAE)

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The aphidophagous hover flies are one of the important group of insect belonging to order diptera, syrphidae syrphinae. The adult are of this group feeds over nectar while the feeding mode of their larvae varies from species to specie. Hence in using floral resources they are very specific as some of them are specialist (feeding over single species of aphids) while other generalist feeding over number of different aphid species. Due to this the adults visit differ floral resources in order arrange the compatible food resources for the growth and proper nourishment of their larvae. The current study was designed to find out importance of these floral resources for the aphidophagous hover flies. In order to study the choosiness behavior different crops, vegetable and ornamental plants of district Raheem Yar Khan were visited from January to May 2018. The visits were made fortnightly in order to get appropriate results. The species of different aphidophagous hover flies were studied in the field and also captured for calculating statistical data. Species were collected with the help of insect net malaise trap and hand picking. Total 346 adults and 34 larvae of 4 species (*Ischiodon scutellaris*, *Episyrphus balteatus*, *Sphaerophoria scripta* and *Sphaerophoria rueppellii*) belonging to three genera (*Ischiodon*, *Episyrphus* and *Sphaerophoria*) were collected from the field. The current finding showed that aphidophagous hover flies were very sensitive in selecting and exploiting the floral resources.

THE POLLUTION OF THE SEA WATER BY THE WASTE FROM THE L.N.G. PLANT ARZEW-ALGERIA AND THE WASTE WATER PURIFYING SYSTEM

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Generally, the pollution make a lot of problems for the environment. The natural gas (N.G.) is a compound of the light hydrocarbons, methane, ethane, propane, butane and other trace elements, like Helium, Nitrogen, Carbon Dioxide and others. After the different zones of the natural gas treatment, a problem of pollution of water is appeared. In this plant there are eight zones, six units of process, one for the energy production and another for the storage. It exists a system to treat all the effluents contamination coming from these zones. The treatment of this waste water consists in the separation of the oil and the sludge to satisfy the standard environmental protection. There are six separators, pools of concrete are connected with a collector for supply which is connected with each separator by three conduits. A diffusion apparatus is put in front of every nozzle of entry in the separator, and this is for a suitable distribution of the liquid in the separator. In reason of the decrease speed of the liquid when it passes through the separator, the particles carried, deposit in the bottom of the separator. The suspended matters are collected by a chain collector. The float matters up the liquid of the separators, mainly oils, are removed through the creaming tubs.

THE EXPERIENCE OF THE P.V. ELECTRIFICATION AND THE SOLAR ENERGY OF SOME LOCALITIES IN THE SOUTH OF ALGERIA

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Algeria, located in North Africa, with an area of more than 2.300.000 Km², 4/5 of it are arid and semi arid zones. This particular situation makes it very costly to extend the conventional energy distribution grid, mainly the villages in Sahara are scattered. Further, the average daily total horizontal radiation calculated over the year is about 6 Kwh/m² for over 3000 hours of sunshine duration. Taking in to consideration this huge renewable energy potential, for this reason, an Algerian governmental solar energy program is retained and a tremendous effort has been done to promote the use of the solar energy. The P.V. electrification of many localities in the south of Algeria is installed. For example: Tin Zaouatine, Ain Guezzam, Bordj Baji Mokhtar, Ain Belbel, Aouelef, Matriouane, et. For example, in a remote saharian village in the south of Algeria, a 6,7 Kwp PV array was installed for the electrification for streets and indoor lighting. The solar energy it is also used in the pumping system for the drinking water and for some needs of the agriculture irrigation. The full solar energy solution has been chosen for the electrification of these isolated regions and to promote the living conditions, such as lighting and other applications. At present the photovoltaic system is running perfectly.

THE STUDY AND THE DEVELOPMENT OF THE RENEWABLE ENERGY IN THE AQUATIC ECOSYSTEM

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The wind, waterfalls, the warmth, the heat of volcanoes, lightning, solar energy captured thanks to solar plates and a lot of qi energy sources are organic and very important, for example the energy of the seas. Ocean energy is renewable energy extracted from the marine environment. The seas and oceans represent 71% of the earth's surface. They could theoretically provide 30,000 SARWG from single solar radiation on the surface which is incredible. An aquatic ecosystem constantly produces living matter. It is gradually transformed into dead organic matter, which is itself then slowly mineralized, in part or in full. The engine of the ecosystem is solar energy; Indeed, only holders of plant chlorophyll pigments are able to capture light energy and transform it in the form of chemical potential energy to manufacture, from minerals, molecules carbonaceous substances such microscopic algae and other. But the ecosystem is a thermodynamically open system, since in fact there are leaks of material therefore energy, following sedimentation and biogeological recycling material, but also due to human samples (fisheries and aquaculture) . Chemical pollutants through the ecosystem and are transferred from one trophic level to the next and are a threat to the ecosystem and ultimately on Hommemais the increase in greenhouse gas emissions are increasing more and this threat affects the habitats and biota and hit the man in fact part.

EFFECT OF INFESTATION BY THE LESSER GRAIN BORER, *RHIZOPERTHA DOMINICA* (F.) ON DIFFERENT WHEAT KERNEL

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The lesser grain borer, *Rhizopertha dominica* (F.), is a serious primary injurious pest of stored wheat in many region of the world. In Pakistan 5-7% of the food grain are lost due to poor storage situation. The adult of lesser grain borer were recorded on three types of wheat, under laboratory condition for some preliminary studies. The Samples

were collected of the different varieties of wheat (Abadgar, TD-1, Moomal) from the Wheat research institute, Sakrand, sindh. During the observation two parameter weight loss and insect damaged grain of percentage with relation to abiotic relation (Temperature and Relative humidity) were seen in the three months April, May, June-2018. During the three month experimentation of *Rhizopertha dominica*, it was notice that Max: weight loss and Insect damaged grain were recorded on wheat variety Abadgar at the average Temp: and R.humidity 35°C, 63% during the June-2018. Their, Min: weight loss and Insect damaged grain were recorded on wheat variety Moomal at the average Temp: and R.humidity 33°C, 50% during the April-2018. Throughout the whole determination its noticed that lesser grain borer more time feeding inside the wheat kernel that is why its quickly reducing the weight and makes them hollow husks. This is the first time study lesser grain borer and wheat varieties from the Wheat research institute, Sakrand, Sindh, Pakistan.

REHABILITATION OF AN INJURED GOLDEN EAGLE (*AQUILA CHRYSAETOS*): A CASE STUDY

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Golden eagle (*Aquila chrysaetos*) belongs to the family *Accipitridae*, is fairly ubiquitous and most widely distributed eagle species, found America, Europe, Africa and Asia including Pakistan. Destruction of habitat causes wild birds to migrate towards urban areas in search of food and sometimes nesting where they are caught by local people by using different techniques and are further used for hunting purposes. An injured golden eagle was found entangled in bushes near the vicinity of Balkasar Research Complex, Chakwal, Pakistan. One leg of the eagles was tied with a rope. Initial screening of the body revealed a wound on the right wing of the eagle, that's why the eagle was not able to fly. The successful treatment was done with anti-parasite and anti-bacterial with careful monitoring. After complete healing of the wound, the eagle was kept under observation for a month and then set free to breath in the open atmosphere. For adaptation of the birds they are usually enclosed in cages or tied with ropes to prevent escaping. In such cases birds try to escape and get injured while colliding with the walls of the cages as observed in current case. The successful treatment and release of wild birds into their natural habitat in such cases can help in their population stability and conservation.

SNAKE BITES CAUSED DEATH OF SEMI-CAPTIVE BROWN BEAR (*URSUS ARCTOS*) AND ASIATIC BLACK BEARS (*URSUS THIBETANUS*): FIRST CASE SERIES FROM PAKISTAN

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Poisoning from snake-bite in animals causes untoward consequences and even death if not treated opportunely. The current paper describes six snake-bite cases over a period of four years (2013-17) in one Brown Bear (*Ursus arctos*) named Lucia and five Asiatic Black Bears (*Ursus thibetanus*) named Shaad, Zoe, Dora, Zorro and Shabnum in a bear sanctuary at Balkasar Research Complex, Chakwal, Pakistan. All the bears were found dead inside the enclosure of the sanctuary during summer season. Eldritch death of healthy bears having no previous health problems or disease history was quite shocking and finding the possible cause of death was necessary for future precautions. Physical examination of the bodies showed sloughing of hair, bluish skin color, ruptured tongue, bluish oral mucosa and abscess on subcutaneous layer of Shaad; swelling in right hind limb, bleeding of dark body fluid, blisters on tongue and upper mucosa of Dora; fang marks on right hind paw and tongue of Zorro which was stiffed and turned black; while in case of Shabnum, bleeding from mouth due to rupturing of tongue, ptosis, sloughing of hairs along with swelling of body and foul odor was observed. The postmortem analysis of Lucia revealed deterioration of lungs while no significant alteration was observed in other organs, while in case of Zoe, edema and renal and pulmonary failure was reported. After considering all relevant factors i.e. environmental and physical, it was concluded that the possible causes of the death in all cases could be snake bites. Wild animals are more prone to snake attack as

compared to domesticated animals. Snake-bites causes untoward consequences and death of the victims. Considerable efforts are required to make epidemiological studies on snake bites and use of anti-venom in farm animals and pets.

DEVELOPMENT OF ELISA FOR NEWCASTLE DISEASE VIRUS (NDV)

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Non-glycosylated matrix (M) protein is one of the abundant structural protein of Newcastle disease virus (NDV) which is the causative agent of Newcastle disease (ND). Newcastle disease is a common infection of many birds including poultry and poses a constant threat to the agriculture and livestock of a country. Considering the conservancy and role of M protein in the viral life cycle, it can be targeted to diagnose NDV. The present study was designed to validate the importance of M protein for developing an indirect enzyme linked immunosorbent assay (ELISA) based study to detect Newcastle Disease in poultry. The recombinant M protein was expressed in *Escherichia coli* and was then purified in its soluble form using the zwitterionic detergent lauryldimethylamine oxide (LDAO). Multiple serum samples were collected from different poultry farms of district Faisalabad. The soluble M protein was then used as the coating antigen in the indirect ELISA for NDV antibodies detection in the samples. The results confirmed the potential role of M protein for NDV detection. This study provides the basis to establish a matrix protein-based indirect ELISA for detection and quantification of the anti-NDV antibody titer in the serum of affected chicken. In order to analyze a large number of samples, the proposed ELISA can be optimized and up-scaled for rapid and reliable detection of NDV infection.

PREVELANCE OF APHIDOPHAGOUS HOVERFLIES (FAMILY: SYRPHINAE SYRPHIDAE: DIPTERA) FROM MANGROVE FOREST HAWKS BAY KARACHI, PAKISTAN

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Pakistan is blessed with inclusive climatic diversity which offers many opportunities for increasing different selection of crops, fruits and vegetables throughout the year. The back-up of crops, fruits and vegetables in Pakistan is essential not only for increasing grower's income and manufacture biodiversity concerns, but also for accumulative the intake of micronutrients in human nutrition through increased crops yield, fruits and vegetable crops consumption. These crops, fruits and vegetable attacked by many notorious insect pest, aphids, jassids, mealy bugs etc. The economic aspects and side effects of insecticides on environment are not neglect able. The environment friendly solution of such type of problems is the use of natural enemies like aphidophagous hoverflies. Natural enemies feed on different insect pests of agro-ecosystems; these relate in complex ways. These bio-control agents may be parasitoids or predators. Relationship between Predator and prey species are one of the best-suited methods in ecology. An ideal natural enemy is one that consumes huge amount of the preys at the right time to maintain an insect pest population below the economic injury inception for the crop production. The family Syrphidae aphidophagous hoverflies subfamily Syrphinae with six thousand described species signifies one of the largest families. Aphidophagous hoverflies are true flies, but they looks like small bees or wasp. They are the helicopter of the insect world, often seen hovering in the air darting a short distance and then hovering again. These beneficial insects are tools in the fight against, aphids, thrips, scale insects and caterpillars. Adults are not predacious, but the larvae prey on many pest. Their larvae may quickly suppress aphids' infestation as each is capable of destroying thousands of aphids colonies during its development stages, where other insects are not in abundant and the larvae of hoverflies usually become the dominant predators on the pest. Mangrove forest sand can be found in the Indus channel and coastline areas of Arabian Sea across the beach Karachi and Pasni in Balochistan. These forests cover an area of 208,000 ha. The mangrove jungle is teeming with different life. They are

natural habitat to a large number of fauna. The present study was carried out from January-March 2018. Total 1038 specimen of one species *Episyrphus balteatus* belonging to genus *Episyrphus* and family syrphinae were first time trapped from mangrove tree(forest) Karachi Hawks bay Karachi, with the help of insect hand net.

INTERACTION OF BIOTIC AND ABIOTIC FACTOR WITH POPULATION OF SYRPHID FLIES IN MIRPURKHAS, SINDH, PAKISTAN

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Syrphid flies provide vital ecosystem services as biological agent and pollinators. They are commonly known as flower flies belong to large family Syrphidae containing about six thousand species worldwide. Presently a detailed survey was conducted in major agricultural field of Mirpurkhas during January to October 2018 in order to monitor the interaction as well as effect of biotic and a-biotic factor with population of Syrphid flies. These flies were trapped by means of two standard methods i.e. Hand sweeping insect net and Malaise trap. As a result a total of 1240 specimens of syrphid flies belonging to 8 species of four genera were collected from the randomly selected crops of studied areas such as brassica, wheat, rice, corn, fruits, fodder and vegetables from different studied localities of Mirpurkhas. The results of this study revealed that both biotic factors i.e. host plants and aphid population was positively correlated whereas temperature, humidity, rainfall and cloudiness had negative correlation with the population of syrphid flies.

PREVALENCE OF TICKS SPECIE *HYLOMMA* AND *AMBLYOMMA* GENERA ON BUFFALO FROM TANDO ALLAH YAR

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A cross-sectional study was conducted in TandoAllahyar district, Sindh, from April 2017 to February 2018 to investigate the prevalence of tick in buffaloes. Dairy farms of five localities namely Bukera Sharif, Dhinghano, Chamber, Nasarpur and Tando Allahyar City were selected for the collection of samples of ticks. Out of the total of 417 buffaloes examined, 389 were found to be infested by one or more tick parasites. A total of 989 ticks were collected from the animal body parts and ticks' genera and species were identified. Total 4 species of 2 genera were identified. From the total ticks collected *Hy. Anatolicum*, followed by *Hylomma excavatum*, *Hylomma scupense*, *Amblyomma americanum*. The age of buffalo showed significant association with the infestation rate. The prevalence of tick infestation was found highest in buffaloes of age 1-3 years (33%) while in buffaloes of age three years it was (16%). The favorable predilection sites of *Amblyomma* species were udder, lower chest and neck. For *Hylomma* species, the perineum region, udder, genital areas and tail were its hiding sites. Prevalence of ticks was highest during summer rainy season from July to October and it was lowest during winter season from January to February.

NEW SPECIES OF A CHEWING LOUSE GENUS *QUADRACEPS* CLAY AND MEINTERZHAGEN, 1938 (PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) FROM LAPWING IN SINDH, PAKISTAN

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During the present study, Red-wattled Lapwing, *Vanellus indicus* (a Charadriiform bird) was collected from different areas of Sindh, and examined for its chewing lice diversity during 2017-2018 for the first time in Sindh,

Pakistan. A total of 100 birds were examined, in which 73 birds were found infested with different species of chewing lice. Among which about 560 specimens were identified as the genus *Quadriceps* Clay and Meinterzhagen, 1938, representative with two species, *Q. dasi* Tandan, 1952 and a new species *Q. sindhiensis* sp.n. the present species was compared with its closest allies within the avian family Charaderiidae, and was found clearly different in having head anterior plates, male and female terminalia, chaetotaxy and male genitalia. The specific name of the species has been given on the type locality from where it has been introduced.

FIRST RECORD OF *DIPLOPHALLUS ANDINUS* VOGEL AND READ, 1953 (CESTODA: ACOLEIDAE), AN AVIAN CESTODE FROM SINDH, PAKISTAN

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For the study of helminthic fauna of aquatic birds, the helminths of Black-winged stilt *Himantopus himantopus* (Linnaeus, 1758) (Charadriiformes: Recurvirostridae) were investigated in Sindh, Pakistan. For this purpose, birds were captured from different localities of Sindh during November 2017 to March 2018. Total 15 birds were dissected for their helminthes parasitic examination, in which four birds were found infected with 24 specimens of *Diplophallus andinus* Vogel and Read, 1953, harbored in small intestine of the host. The cestodes were recovered alive from hosts, provided with excellent stained material and preserved permanently in Canada balsam. Scolex bears four suckers and well developed rostellum without hooks. Strobila is gradually broadens, thick and posterior to short neck with maximum width attained with semi gravid. The internal organs first appeared a few millimeters posterior to scolex. These cestodes were recovered from *Himantopus himantopus* for the first time in the region, hence making new host as well as new locality record for this species of cestodes from Pakistan.

NEW RECORDS OF PHTHIRAPTERAN ECTOPARASITES FROM *COTURNIX JAPONICA* (GALLIFORMES: PHASIANIDAE) IN SINDH, PAKISTAN

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Coturnix, commonly known as Quail or Batair is very popular and economically important bird of avian order Galliformes, family Phasianidae and subfamily Perdicinae, found in Sindh Pakistan. It is used for both as pet and farming purposes. *Coturnix* has 08 species in the world, amongst which 04 are found in Pakistan. However two species, *C. coturnix* and *C. coromandelica* are native in Sindh. *Coturnix* is affected from different types of parasitic diseases among which lice infestation is considered as the most prevalent infestation. This is the first ever study on the ectoparasites of *Coturnix* in Sindh, Pakistan. The study revealed the presence of ectoparasites on *Coturnix* followed by the survey and the collection of *Coturnix japonica* from different districts of Sindh. All the collected birds were inspected for the ectoparasites. The prevalence of ectoparasites on *Coturnix japonica* was found 100% from all the collected 40 birds. The collected lice were preserved in 70% ethanol, prior to slide preparation using KOH and passing from ethanol series. The species were mounted in Canada Balsam using standard procedure for the identification of species ahead. In the present study, one species of the family Philopteridae (Ischnocera) named *Cuclotogaster cinereus* (Nitzsch, 1866) was evidently found abundantly among the all other collected species of lice such as *Goniodes astrocephalus* (Burmeister, 1838) and, *Menacanthus abdominalis* (Piaget, 1880), belonging to family Menoponidae (Amblycera). All of these species have been reported for the first time from Sindh region, making new locality and new host record from Sindh, Pakistan.

A SURVEY OF CHEWING LICE (PHTHIRAPTERA: AMBLYCERA AND ISCHNOCERA) ON MIGRATORY BIRDS IN SINDH, PAKISTAN

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During present study, a variety of migratory birds were examined for their chewing lice diversity in Sindh province, Pakistan during the years 2017 and 2018. In the survey the birds were collected, belonged to family Anatidae were common teal (*Anas crecca*), shoveler (*Anas clypeata*), mallard (*Anas platyrhynchos*), common pochard (*Aythya ferina*) (Anseriformes); family Rallidae were Eurasian coot (*Fulica atra*), common moorhen (*Gallinula chloropus*), family Gruidae was Common Crane (*Grus grus*) (Gruiformes); family Ardeidae was Black-crowned Night Heron (*Nycticorax nycticorax*) (Pelicaniformes); family Phoenicopteridae was Lesser flamingo (*Phoenicopterus minor*) (Phoenicopteriformes); family Laridae was Black-headed Gull (*Chroicocephalus ridibundus*) (Charaderiformes). These birds were brought to APRL at Department of Zoology University of Sindh, Jamshoro, birds were examination for their chewing lice through fumigation and visual examination methods, the recovered specimen were dehydrated in graded series of ethanol and mounted permanently through standard method. Among 90 birds, 76 were found infested with chewing lice having 84.40% prevalence. In present, 13 species of chewing lice of family Menoponidae and family Philopteridae were recovered and identified as *Anatoecous icteroides* (Nitzsch, 1818), *Anaticola crassicornis* (Scopoli, 1763) *Trinoton querquedulae* (Linnaeus, 1758) and *Holomenopon leucoxanthum* (Burmeister, 1838) on anatids bird; *Fulicoffula luridae* (Nitzsch, 1818) and *Pseudomenopon pilsom* (Scopoli, 1763) on eurasian coot and common moorhen; *Heleonomus macilentus* (Nitzsch 1866) on cranes; *Ardeicola cruscula* (Carriker, 1960) and *Ardiphilus vittatus* (Rudow, 1866) on night herons; *Austromenopon himantopi* (Timmermann, 1954) and *Lunaceps limosella* (Bechet, 1968) on black-headed gull, *Colpocephalum heterosoma* (Piaget, 1880) and *Anaticola phoenicopteri* (Coinde, 1859) on lesser flamingo. This was the first survey of parasitological parameters related to chewing lice was undertaken in the Sindh region, Pakistan.

PREVALENCE OF CHEWING LICE (PHTHIRAPTERA: AMBLYCERA AND ISCHNOCERA) ON *ACRIDOTHERES TRISTIS* (PASSERIFORMES: STURNIDAE) FROM SINDH, PAKISTAN

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Survey of literature showed that the population characteristics of the phthirapterans parasitizing on *Acridotheres tristis* (Common Myna) deserved analysis. During present studies common mynas were collected from different localities of Sindh, Pakistan. Birds were collected during 2014–2016 by trapping and were brought to the Advanced Parasitology Research Laboratory (APRL), Department of Zoology, University of Sindh, Jamshoro for further studies. Myna kept in a Fumigation Chamber for their chewing lice for at least 1 hour, which may give a result of maximum number of ectoparasites by which four species of four genera were recovered. Two species of family Menoponidae were *Menacanthus eurysternus* (Burmeister, 1838), *Myrsidea ahmedalii* Bughio *et al.*, 2018 and two species of family Philopteridae, *Sturnidoecus tristisae* Bughio *et al.*, 2018 and *Brueelia sindhiensis* species novum. Total prevalence of chewing lice was found 100% on all infested birds of *Acridotheres tristis*. The chewing lice species wise parasitic infestation was found maximum for *Myrsidea ahmedalii* (59.36%) and *Sturnidoecus tristisae* (39.5%), while moderate infestation was found by *Menacanthus eurysternus* (36.5%) and *Brueelia sindhiensis* (36.4%).

**RECORD OF THE GENUS *CARDUICEPS* CLAY AND MEINERTZHAGEN, 1939
(PHTHIRAPTERA: ISCHNOCERA) FROM BLACK-WINGED STILT (CHARADRIIFORMES:
RECURVIROSTRIDAE) IN SINDH, PAKISTAN**

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Fantastic study was conducted on chewing lice (Phthiraptera) on black-winged stilt, *Himantopus himantopus* (L.) of family Recurvirostridae from Sindh Pakistan. During Oct, 2017 to March, 2018, total of thirty five hosts were collected from different localities of Sindh province, and examined for their lice in which all were found infested with more than 200 specimens of the genus *Carduiceps* Clay and Meinertzhagen, 1939 were recovered. The genus is first time reported from the present host from Sindh, Pakistan hence, making new locality and new host record.

**OCCURRENCE OF SCHIZAPHIS GERANIIUM AND RHOPALOSIPUM PADI APHID SPECIES
(HOMOPTERA: APHIDIDAE) ON WHEAT IN JACOBABAD, SINDH, PAKISTAN**

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Wheat *tritium aestivum* L is leading food grain and staple diet in Pakistan it contributes 18% value added in agriculture and 2.4 in GDP. It is cultivated in an area of 8578 thousand hectares with production of 21% million metric tons per year. Beside it wheat is attacked by various pests, which caused serious damage and lead reduction of yield. *Schizaphis granium* and *Rhopalosipum padi* (Homopterous: Aphidian) are serious pests of wheat. Present study was carried out from January to December 2017. We have surveyed different fields of Jacobabad and collected the aphids from various grasses of wheat and wheat plants. They have been brought to the laboratory into plastic jars and preserved in Glycerine. Total 2028 specimens were collected from both species, but *Rhopalosipum padi* remains high in infestation rate with total 1026 specimens than *Schizaphis granium* which had 1002. We collected aphids in colonies. The present study revealed that serious infestation of this pest may demand, wide use of insecticides to save the infested crops.

DIVERSITY AND COMPOSITION OF WATER BIRDS AT RIVER KUNHAR IN KPK, PAKISTAN

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The total length of River Kunhar is 166 Km, originates from Lulusar Lake below Babusar Top. The water level of the river expands in summer and shrinks in winter. The present study was carried out to determine the composition and diversity of the water birds, total water birds species, residential and migratory birds at river as well as factors responsible for their habitat disturbance. Shohal, Batsing Baila and Garhi Habibullah were the study sites for our survey. Interview was conducted from the wildlife staff, residents, and direct method of observation by using binoculars was adopted for the collection of data. Among birds species, adapted to diverse habitat at the river Mallard Duck (*Anas platyrhynchos*), White Stork (*Ciconia ciconia*), Swan (*Cygnini*), Crane (*Grus grus*), White-breasted waterhen (*Amaurornis phoenicurus*) and Kingfisher (*Alcedo atthis*) were present (observed early in the morning) which probably were migrated from the Siberia over there. Among all, Mallard Duck was dominant water bird. All these water birds prefer the habitat at river shores but *Mallard Duck (Anas platyrhynchos)* is the only bird which lives

inside the river. Hunting and fishing are the two main causes of their disturbed habitat which lead to the extinction of many waterbirds species. Finally all the data provided strong argument to the preservation of these waterbirds by keeping proper check and balance over hunting and fishing at the river.

STUDY OF HAZARDOUS EFFECTS OF ZINC OXIDE NANOPARTICLES WITH *DROSOPHILA MELANOGASTER* AS A MODEL ORGANISM

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The nanoparticles as remarkable tools in biological and medical science made quite an impression, but they can have some toxic effects due to their complex nature, like the ZnO nanoparticles (ZnO-NPs). Zinc is an essential trace metal in a living body but concentration above optimum can cause intensive damage, while this nanotoxicity can be applied in controlling parasites and pests. In current study, *Drosophila melanogaster* was used as a model to study the ZnO-NPs effect on fatality, behavior, developmental delay, wings morphology, abdominal pigmentation and fecundity by exposing larvae and adults to small, spherical nanoparticles, after preparing with *Citrus limon*, while characterization performed via Ultra-violet vision (UV-Vis), Fourier-transform infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM) and X-ray diffraction (XRD). Various concentrations of ZnO-NPs (0.5, 5, 10, 16 and 20 mM) were added into food media, applied for 24-hour and 4 days to larvae and adults, respectively. Toxicity was found to be highly significant at 16 and 20 mM with 43.33%-51.66% (larvae) and 60%-66.67% (adults) fatality, lowering speed to 2.4 cm/min and 1.76 cm/min in larvae crawling behavior, 39.47-2.62 hours developmental delay and 2.875-5.25 g weight lessening. High deviations in wing morphology and lightening pigmentation was marked. The fecundity at 20 mM halved (148 to 70) from that of control. The effect was found to be augmented with rising concentration and adults were less viable than larvae. ZnO-NPs at higher concentrations are substantially toxic, so, quantity of these nanoparticles in human products should be monitored. The further studies were recommended.

DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES IN KARACHI, ITS STORAGE AND EFFICACY AGAINST SOME INSECT PESTS

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The present study was based on Distribution of *Steinernema ceratophorum* from different fruits, vegetables in Karachi and its storage and efficacy against some insect pests. Out of 50 soil samples collected from 20 different localities of Karachi, Entomopathogenic nematodes were detected from 5 samples, out of these 3 isolates of *Steinernema ceratophorum*, *S. pakistanense*, *S. simkayai* and 2 of *Heterorhabditis indica*. *S. ceratophorum* was stored on different substrate to check the shelf life of nematodes and tested against different insect pests under laboratory condition.

STUDY OF BIODIVERSITY OF INTERTIDAL ZONE OF BULEJI, A ROCKY SHORE OF KARACHI COAST (NORTHERN ARABIAN SEA)

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Pakistani coastal waters is blessed with a variety of rich biodiversity of marine fauna. Buleji, (Karachi) is well known to be one of the expedient locations to study the rocky marine intertidal fauna of Pakistan. The study was

analyzed along two of 540 and 570m long sublittoral transects. The study is comprised of two parts, the present quantitative assessment of invertebrate fauna and a comparison with the earlier studies. There is rich and vast fauna of Molluscs in higher dominance especially gastropods, bivalves and univalves. A large number of Crustaceans represented by crabs of different families, marine shrimps, annelids, few cephalopods (*Octopus*) and Echinoderms are also recorded during the study. The comparison of the fauna with earlier studies will be a useful tool to monitor the health and status of animals in monitoring the impact of increased civilization and pollution on marine life in Buleji.

PREVALENCE OF VARIOUS PATHOGENIC VARIANTS RESPONSIBLE FOR EARLY INFANTILE EPILEPSY AMONG PAKISTANI POPULATION

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Epilepsy is the neurological disorder that indicates abnormal activity in central nervous system. It is caused due to genetic as well as non genetic factor. Prevalence rate is higher in non developed countries. In Pakistan the prevalence of epilepsy is about 9.99/1000. Nearly 50 million people in whole world suffer from epilepsy. Different risk factors such as head trauma, central nervous system infections, poverty and tumors are associated with development of epilepsy in different age groups. We studied patient facing epilepsy in early age. The objective of this study was to range of genetic variants that can affect the risk for causing epilepsy in Pakistan. Data was analyzed through whole exome sequencing. More than 1500 genes involved in epilepsy. Mutations in some genes caused epilepsy and seizures. Voltage gated sodium as well as potassium and calcium channels have significant role in epilepsy. SCN1A is the major cause of epilepsy but SCN2A and SCN3A have been associated with epilepsy. Mutation in SCN8A gene causing infantile epileptic encephalopathy and is associated with sudden unexpected death due to epilepsy. CHRNA4 is the first epileptic gene. This is responsible for autosomal dominant nocturnal frontal lobe epilepsy. Mutation in SCN1A and protocadherin 19 (PCDH19) associated with dravet syndrome. Mutations in KCNQ2, KCNQ3, RBFOX1 and SRPX2 associated with rolandic epilepsy. Juvenile myoclonic epilepsy is severe form of epilepsy that is started between age 6- 36 years. Its symptoms begin at age 12. It has generalized tonic clonic seizures. Mutation in EFHC1 associated with juvenile myoclonic epilepsy. A miss sense mutation identified in this gene under snip rs3804506. Amino acid change take place at position Arg159Trp and nucleotide change at 475C>T. We show that miss sense pathogenic variants are associated with development of epileptic encephalopathies. This gene play significant role in juvenile myoclonic epilepsy.

TAXONOMY AND ECOLOGY OF HEDGEHOG GENUS *HEMIECHINUS*, (EULIPOTYPHILA, ERINACEIDAE) OF DISTRICT JAMSHORO, SINDH, PAKISTAN

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The *Hemiechinus* Fitzinger, 1866 Hedgehogs include about 16 species around the world. They are distributed in Europe, Africa and Asia. The literature reveals that 04 species of Hedgehog inhabits the plains of Pakistan adapted to xeric/desert ecosystem specially the Aerial plains of Indus river which extend to words east up to Cholistan and Thar, including Bahawalpur as well as irrigated areas of Punjab. Ecologically Hedgehogs mostly inhabit agricultural, rocky and steppe zones and wherein they showed their presence in variable temperatures such as 20 °C to 40 °C. Being omnivorous feed on insects, butterfly, meat and lower mammals etc. During the present studies total of 107 specimens have been recorded, among them only 05 different species of Hedgehog have been identified viz: 25 species of *Hemichinus collaris* (09 ♀ and 16 ♂); 25 species of *Hemichinus auritus* (10 ♀♀ and 15 ♂♂); 22 species of *Paraechinus hypomelas jerdoni* (12 ♂♂ and 10 ♀♀); 15 species of *Paraechinus hypomelas hypomelas* (09 ♂♂ and 06 ♀♀) and 20 species of *Paraechinus micropus* (13 ♂♂ and 07 ♀♀) respectively. It was observed that the

prevalence of the *Hemiechinus collaris* species (23.364%) and *Hemiechinus auritus* species (23.364%) was highest followed by the *Paraechinus hypomelas Jerdoni* species (20.560%) and *Paraechinus mcropius* species (18.691%) respectively. The minimum prevalence was found for the *Paraechinus hypomelas hypomelas* species (14.018%).

PREVALENCE OF MEALY BUGS (HOMOPTERA) FROM VARIOUS LOCALITIES OF HYDERABAD DIVISION

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Various localities of Hyderabad was surveyed carried from April to June 2018. In this survey three species of Mealy bugs belonging to three genera were collected from Hyderabad, *paracoccus marginatus* (60%), *phenococcus selonopsis* 30% *planococcus kenyae* (10%) Incidence of mealy bugs were increased in monsoon from July to September.

ABUNDANCE OF THREE SPECIES OF TWO DIFFERENT GENERA OF SUB-FAMILY APHIDINAE (HOMOPTERA: APHIDIDAE) IN JAMSHORO, SINDH, PAKISTAN

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Aphids are polyphagous insect belonging to order Homoptera family Aphididae. Many species of this group are major pest of cotton, brassica, vegetables and many fruits. Present study was conducted from September 2018 to December 2018 in different agricultural field of Jamshoro. Aphids were collected randomly from the plants through different methods (1) hand picking (2) by using soft hair camel brush and (3) jarring the plants on white paper sheets. After collection, the specimens were brought to Entomology laboratory department of Zoology for further observations. Total 1229 colonies of 03species *Myzus persicae*, *Aphis neri* and *Aphis fabae* belonging to two genera and one sub family Aphidinea of Aphididae family were observed *Myzus persicae* was found most abundant species and observed on 05host plants throughout study period, total 533 colonies of *Myzus persicae* were observed second abundant species was *Aphis fabae* recorded from 03 host plants while *Aphisnerii* was found limited to particular host and season. During present study 09 host plants were found positive against aphid infestation (brassica, wheat, spinach, Grasses, chili, cabbage, akk, oleander and ornamental plants).

THE QUANTITATIVE EVIDENCE OF MALARIAL TRANSMISSION IN DIFFERENT AGE GROUPS FROM DIFFERENT LOCALITIES OF SHIKARPUR, SINDH, PAKISTAN

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Malaria transmission is an extremely complex condition that is manifested differently in different parts of the world. In Pakistan, malaria is still endemic in many areas. *Plasmodium vivax* and *Plasmodium falciparum* are identified to be the most prevalent species of malaria in Pakistan. . Present study was conducted from January 2018 to December 2018 in different localities of district Shikarpur. The data were collected from health facilities, the parasites were identified by using Giemsa stained thick and thin smears prepared from suspected patents of fever. A total of 2269 blood smears were prepared from the age groups ranging from 1 year to 14 years in 3 different localities district .Out of 1050 patients 21 were found positive, in the age group of 1-5 years,(2178)cases were found in the age

groups of 5-14 years among them 45 were reported positive,(1176) cases were found above 14 age groups among them 39 cases were reported positive.

SEASONAL CHANGES IN MALARIA EPIDEMIOLOGY IN DISTRICT SANGHAR, SINDH, PAKISTAN

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Malaria is a dangerous disease caused by Plasmodium species and transmitted by female Anopheles mosquitoes. Half of the world population is at risk of malaria; Pakistan is listed among moderately prevalent countries for malaria. In Pakistan two species of malarial parasite are widely distributed i.e. *P. vivax* and *P. falciparum*. Mosquitoes are the vector of malaria in which *Anopheles culicifacies* and *Anopheles stephensi* are important vectors of malaria in Pakistan. Meteorological parameters affect the population of mosquitoes and malaria burden i.e. temperature, humidity and rainfall. The Present study is carried out during April 2017 to December 2017 in three talukas (Tando Adam, Shahdadpur and Sanghar) of district Sanghar. Data of malaria were collected from taluka hospitals of different localities of district Sanghar. Total 18206 suspected cases of malaria were examined. Among these cases, 100 malaria positive records were found from April to June, 192 malaria infections were found after the rainy season during July to October while 59 cases in extreme cold weather i.e. November and December. According to above mentioned record more prevalence of malaria was found in post monsoonal season during July to October.

THE EXPERIMENTAL WORK ON DIFFERENT PLANTS EXTRACTS WHICH WAS USED AS BIOPESTICIDE TO CONTROL THE OKRA JASSID (*AMRASCA DEVASTANS*) (DIST.) IN LAHORE PUNJAB

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A field experiment was carried out to evaluate the efficacy of bio-pesticides against jassid on okra so that best biopesticide can be used for kitchen gardening in homes which is safe for environment and human health. The experiment was conducted at Institute of Agricultural Sciences University of the Punjab Lahore. The bio-pesticides were applied against jassid at economic threshold level. Treatment applied Neem extract, Datura plant, Tobacco extract, Eucalyptus and Aloe vera plant extracts. Pre-treatment population of jassid was recorded one day before spray from randomized selected 5 plants from each treatment. While post treatment jassid count was made after 24, 48, 72 hours one and two weeks of spray. Neem extract (600ml), Datura plant, (600ml), Ginger leaf extracts (600ml), Eucalyptus (500ml), aloe vera plant (500ml) were used as biopesticide. The randomized complete block designs with 4 replications were used. The data were recorded at weekly based and all the agronomic practices were used. The 15 percent stock solutions from plants leaves extract were used. The maximum control were showed by the neem leaves extract and population were noted at that treatment was (0.88/leaf). The maximum population of jassid were found on the Eucalyptus leaves extracts treatment which was 3.23/leaf. If we discuss the result of other treatments like tobacco extracts, Datura plant extract and aloe vera plant showed following results, 1.01/leaf, 1.69/leaf and 2.55/leaf respectively. To seeing the result we can conclude if neem can be used at home level it can control the jassid and also other sucking insect pest of okra. The data was analyzed on an IBM-PC Computer using M Stat Package.

SILK SERICIN– A PROMISING AGENT FOR TREATMENT OF CHRONIC WOUNDS

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Wound healing is a complex, highly regulated process that is essential in maintaining the barrier function of skin. With numerous disease processes, such as diabetes, the orderly progression of events involved in wound healing can be affected, resulting in chronic, non-healing wounds. Scientists have tried various approaches to help chronic wounds heal, including bandaging, dressing, exposure to oxygen and growth factor therapy, but they often show limited effectiveness and also have certain limitations. Silk protein sericin, acquired from silkworms *Bombyx mori*, possess hydrophilic properties, compatibility with skin and biodegradation, hence considered as an excellent wound healing agent. The present study was carried out to evaluate the effects of sericin on wound healing in mouse model by creating full thickness excision wounds on the dorsum. Group 1 animals were considered as negative control, therefore no treatment was given in this group. Wounds were simply cleaned with sterile normal saline solution daily. Group 2 animals were treated with glycerine and Group 3 animals were treated with 5% sericin and glycerin formulation. Wounds treated with 5% sericin and glycerin formulation showed rapid and effective healing within 11 days, and also showed much smaller inflammatory reactions. However, wounds of animals in Group 1 and Group 2 showed healing in 20 days and 15 days respectively. Some acute allergic reactions were also observed in Group 2 animals. All these results indicate that topical application of sericin has wound healing effects without causing allergic reactions and it can be considered as a better candidate for treatment of chronic wounds.

EPIDEMIOLOGY OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN DIABETIC PATIENTS

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Among all the pathogens discovered, *Staphylococcus aureus* dominates in patients suffering from diabetics. It is considered that the prevalence of methicillin resistant *S. aureus* (MRSA) is 15-30% in diabetic infectinos mainly targeting foot wounds and skin ulcers. Diabetic Foot Infection (DFI) is common amongst diabetic patients in Pakistan. Many cases of MRSA have been surfaced in the last decades in Pakistan. The purpose of this study was surveillance of MRSA and to determine the most common drug resistance against *S. aureus* in diabetic patients. Data of diabetic patients was collected from Sheikh Zayed Hospital, a retroactive examination of total 175 samples were studied during December 2017 to May 2018 and screened for MRSA using mainly cefoxitin blood agar and oxacillin resistant screening base agar (ORSBA). Specimens were cultured considering the microbiological techniques which are recommended by Clinical and Laboratory Standards Institute (CLSI) along with performance of antibiotic susceptibility tests. Disc diffusion point assay was used to measure sensitivity of isolated patients while to find out the minimum inhibitory concentration (MIC) of various antibiotics, broth dilution process was performed. Isolates were also tested against commonly used antibiotics. The colonies were mostly found in the foot site. To isolate MRSA, ORSBA was preferable over the other agar medium. Major risk issues for colonization of MRSA were male patients. Isolated patients were responsive to vancomycin and linezolid. Most of the isolates were also responsive to rifampicin. High level of resistance was shown by penicillin and ampicillin. Measures like better hygiene and proper use of antibiotics should be adopted to cure the infection.

EFFECT OF HEXANE FRACTION OF *CISSUS QUADRANGULARIS* ON THE DIFFERENTIATION AND MINERALIZATION OF MOUSE PRE-OSTEOBLAST CELL LINE MC3T3-E1 (SUB-CLONE 4)

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Cissus quadrangularis (CQ) commonly known as bone setter is a shrub of Vitaceae family of plants and is found in southeast and far eastern countries including Pakistan. It has been reported for its medicinal properties in ancient and modern literature but it is most famous for its bone healing properties. The present study is part of a project that explores the osteogenic potential of *Cissus quadrangularis* (CQ). Crude Ethanolic extract of CQ was prepared and fractionated into four fractions i.e. n-Hexane (CQ-H), Dichloromethane (CQ-D), n-Butanol (CQ-B) and Ethyl Acetate (CQ-E). The present study reports the effect of CQ-H on the differentiation and mineralization of MC3T3-E1 cell line. Growth curve, proliferation and viability assays revealed 1ng/ml - 100ng/ml CQ-H as the non-detrimental doses for CQ-H. MC3T3-E1 cells were induced to differentiate into osteoblast cells in presence of osteogenic medium for 21 days. The non-detrimental doses of CQ-H were tested against positive and negative control for their effect on differentiation and mineralization of MC3T3-E1 cell line. Enhanced and early mineralization was observed in presence CQ-H as compared to positive control. The results are supported by histochemical staining (VonKossa staining, Alizarin Red S staining and ALP staining) of differentiated cells and the expression profile of osteoblast marker genes such as Runx2, Osterix, Collagen, Alkaline Phosphatase, Integrin related Bone Sialoprotein, Osteopontin and Osteocalcin. These findings suggest that CQ-H enhances the differentiation and mineralization potential of MC3T3-E1 cells into osteoblasts, thus indicating its potential to treat bone related problems. Further investigation is going on to identify the active compounds and understand their effect on the differentiation and mineralization of MC3T3-E1 cells up to molecular level.

DETERMINATION OF MITOCHONDRIAL DYSFUNCTION DURING DIFFERENTIATION OF MOUSE PRE-OSTEOBLAST CELL LINE MC3T3-E1

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Mitochondria are powerhouse of a eukaryotic cell and are involved in energy generation by process of oxidative phosphorylation. The proteins participating in ETC are nuclear and mitochondrial encoded. In addition to energy production, this process also cause production of reactive oxygen species (ROS) and associated diseases. There is a strong link between dysfunctioning mitochondria and aging associated phenotypes. Due to decline in function of mitochondria, ROS generation elevates with advancing age and the activity of ROS-eliminating enzymes decline as aging progresses. Mitochondrial DNA mutations accumulate in aging that impair the normal functioning of ETC. This cause elevated levels of ROS to be generated and cause serious damage to DNA, lipids and proteins. The aim of this study is to determine the mitochondrial dysfunction during differentiation of bone cells. MC3T3-E1 is mouse pre-osteoblast cell line. This study is based on differentiation of MC3T3-E1 cells. This differentiation was confirmed by histochemical stainings and by real time PCR analysis of bone marker genes i.e. ALP, Runx2, SP-7, IBSP, OPN, OCN and Col1a1. The mitochondrial dysfunction during differentiation of bone cells was determined by real time PCR analysis of marker genes i.e. Tfam, CHOP, ATF-3, ATF-4, ND-1, ND-4, Sirt1 and Sirt3. The expression of mitochondrial dysfunction genes during differentiation of bone cells was very interesting, with expression of ND-1 and ND-4 decreasing, expression of Sirt 1 and Sirt 3 increasing and expression of ATF -3, ATF-4 and Tfam increasing and then decreasing during differentiation. This study generated quite accurate results due to the use of real time analysis and it hold promise for completely understanding the function of different mitochondrial dysfunction markers for their use in therapeutics.

MORPHOMETRIC RELATIONSHIP OF TWO COMMERCIAL FISH SPECIES OF FAMILY SCIAENIDAE (*OTOLITHES RUBER* AND *O. CUVIERI*) ALONG THE PAKISTAN COAST

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The coastal waters of Pakistan are rich in many commercially valuable finfish and shellfish fisheries. Among the fin fish, Sciaenids are commonly known as drums and croakers and have a marked economic importance in local and international market with substantial value. The family Sciaenidae recognized as an important fishery resource worldwide, with about 70 genera and 270 species, but there is little information available on the taxonomy and biology of Sciaenids in Pakistan. Thus, the current study was designed to evaluate the range, variation and relationship in morphometric parameters; such as total length (TL), standard length (SL), head length (HL), dorsal fin's length (DFL), pectoral fin's length (PFL), caudal fin's length (CFL), body width (BW) and body wet weight (Ww) of two commercial species, *Otolithes ruber* and *Otolithes cuvieri*. The mean total length and standard length (TL = 36.73 ± 73.0 , SL = 31.70 ± 6.20) of *O. cuvieri* was greater than *O. ruber* as were assessed (TL = 25.38 ± 2.71 , SL = 22.11 ± 2.44) accordingly. The Pearson's correlation analysis was applied between morphometric parameters of both species and the total length was positively correlated ($p < 0.05$) with all other parameters. The linear regression coefficient (R^2) revealed the body parts of both fishes followed an isometric growth pattern.

SOME OBSERVATIONS ON MORPHOLOGY AND HISTOLOGY OF VENOM GLAND OF SEA SNAKE *HYDROPHIS CAERULESCENS* FOUND IN SONMIANI WATERS, BALUCHISTAN, PAKISTAN

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Sea snakes are an exceptional characteristic of marine environments of Pakistan. Its availability and abundance indicates distinctive marine ecosystems of the Arabian Sea. On the entire coastline, Sonmiani has been found to be a hot spot of species' availability and abundance of sea snakes. Out of all, *Hydrophis caeruleus* is one of the most abundantly found species as bycatch after *Hydrophis schistosus* in Sonmiani and is of interest because of its lean body and agile behavior. It is found in shallow waters and is easily spotted on the clear surface waters. The body size and shape of the head clearly indicated the predacious nature of this species and therefore, an important part of the lagoon food chain. It mostly feeds upon prey smaller in size than itself, by using its neurotoxin venom as a weapon and this mechanism functions by fang, venom gland and its venom case that is encapsulated in the cephalic region behind eyes inside the skull. Venom glands are paired structures attached to the lower mandible for support and upper mandible to a pair of hollow fangs. The mouth also consists of rows of non-venomous teeth. Fangs and teeth are directed inwards and venom glands are globular in shape. The observations included histological components and remarks about the structure of the venom gland. It is found to be a simple gland with linings of secretory epithelial cells and a lumen encapsulated in a muscular sheath.

INFLUENCE OF SOCIAL CONTEXT ON NOVEL FOOD PREFERENCE BY GREAT TIT (*PARUS MAJOR*)

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Throughout the animal kingdom, individuals often differ consistently from one another in the way they cope with the novel and challenging environment. These consistent individual behavioral differences across time and

context are called personality. In particular, personality is a substantial driver of a range of important ecological and evolutionary processes. As most animal species are social for at least part of their lives, and group living is common. A key link between personality and the social context may be expected, however, most personality studies have investigated consistent individual behavioral types and their consequences in asocial contexts. But what is the role of personality in collective behavior and group functioning remain poorly understood. We chose Great tit (*Parus minor*) a social model species, as subject to test the personality traits, and the integrated link between personality traits and social context. We classified individual personality by observing their exploration behaviors in a novel room, after observing individuals with varying personality types: both fast and slow explorers individuals were presented with different novel food in asocial and social context. We found that during asocial context fast explorer took less latency to eat peanuts than bread, made more visit to the feeder, and spent greater time at the feeder, while slow explorer took less latency to eat sunflower seeds than popcorn. However during social context slow explorers preferred peanuts like fast explorer individuals. These results showed that individuals with different personality type might influence each other's foraging behavior under social context. This change in individual behavioral performance in the presence of conspecifics can enhance social stability and develop the functioning of social groups in wide range of contexts.

HISTOLOGICAL RESPONSES IN INTESTINE, KIDNEY AND LIVER TISSUES OF *LABEO ROHITA* DURING ACUTE AND CHRONIC EXPOSURE OF PESTICIDE, CHLORPYRIFOS

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The aim of present study was to examine the acute and chronic exposure of pesticide, chlorpyrifos (CPF) to the fresh water fish *Labeo rohita*. During acute exposure, fish were exposed to different concentrations of CPF ranging from 0, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L for 96 hrs in glass aquaria. The 96 hrs LC₅₀ value of CPF for *Labeo rohita* was found to be 0.01 mg/L. During chronic exposure fish were subjected to 1/3rd, 1/5th, 1/7th and 1/9th of LC₅₀ for 30 days. At the end of the trial, tissues from various organs like intestine, liver and kidney were collected and sections were observed in digital microscope. The pronounced histological changes like necrosis, infiltration, atrophy, shrinkage and degeneration of intestine was observed in the intestine at different CPF concentrations. The kidney sections of *Labeo rohita* under different CPF concentrations exhibited nuclear hypertrophy, vacuolar degeneration of glomeruli, and occlusion of tubular lumen, cloudy swelling degeneration and hyaline droplets degeneration. In the liver tissue of *Labeo rohita* prominent histological changes including hepatic cell degeneration, nuclear hypertrophy, bile stragnation, irregular shaped cells, degeneration in the liver parenchymal cells, nuclear and cytoplasmic degeneration were observed. It is therefore concluded that Chlorpyrifos adversely affects the major organs of the fish *Labeo rohita*.

CHARACTERIZATION OF CELLULOSE DEGRADING BACTERIA FROM *HETEROTERMES INDICOLA*

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The present study involves the isolation of actinomycetes from the gut of *Heterotermes indicola*. Morphological identification (colony size and shape) was used to identify these as actinobacteria. Maximum bacterial growth was observed at optimum temperature 37°C, pH 7 and inoculum size 2%. Biochemical tests including Gram staining, oxidase test, catalase test, indole test were positive for these bacteria. Congo-red method confirmed cellulolytic ability of these bacteria. A total of 3 cellulose degrading bacteria (CDB) were isolated on the basis of radiance zone formation. Maximum hydrolysis capacity for carboxymethylcellulose was from 0.8cm -1.0 cm. The cellulase assay

showed its activity from 20.298U/ml/min to 93.298 U/ml/min. Nitrate reduction assay proved their nitrogen fixation ability.

**A STUDY ON EUPHLYCTIS IN SELECTED AREAS OF KARACHI WITH
REFERENCE TO HABITAT DESTRUCTION**

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The investigations were carried out to determine the effects of habitat destruction on Euphlyctis in selected areas of Karachi District. During the survey, in all the selected areas, only one species was found, i.e., Euphlyctis cyanophlyctis (Common Skittering Frog). The study areas included temporary water pools and permanent water bodies. The geographical coordinates of all these habitats were also recorded. It was observed that some of the temporary water pools dried out after three days of rainfall and due to habitat destruction most of the tadpoles were predated by other organisms. Whereas some permanent water bodies were neither destructed, nor altered during the studies. Adult Euphlyctis cyanophlyctis (Common Skittering Frog) were mostly observed in summer season and after precipitation. The present study indicated that the population of Euphlyctis cyanophlyctis (Common Skittering Frog) is facing a serious threat towards its elimination from the environment due to habitat destruction. Major causes of habitat destruction are change of climatic conditions, urbanization of land, lack of sufficient rainfall and improper disposal of waste. It was found that there are only a few numbers of wetlands in Karachi District that serve as good habitats of Euphlyctis cyanophlyctis (Common Skittering Frog) so there is a serious need of conservation and management plans for the protection of common skittering frog.

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