

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

**Volume 36, 2016**

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



**THIRTY SIXTH PAKISTAN CONGRESS OF ZOOLOGY**

*held under auspices of*

**THE ZOOLOGICAL SOCIETY OF PAKISTAN**

*at*

**DEPARTMENT OF ZOOLOGY, UNIVERSITY OF SINDH,  
JAMSHORO**

**FEBRUARY 16 – 18, 2016**

# PROCEEDINGS PAKISTAN CONGRESS OF ZOOLOGY

(Proc. Pakistan Congr. Zool.)

## CONTENTS

	Pages
Acknowledgements	i
Programme	ii
Members of the Congress	xi
<b>Citations</b>	
<b>Life Time Achievement Award 2016</b>	
Prof. Dr. Muhammad Saeed Wagan .....	xiii
Mr. Zahid Beg Mirza .....	xiv
Dr. Abdul Aleem Chaudhary.....	xv
Prof. Dr. Afsar Mian .....	xvii
Prof. Muhammad Arslan.....	xiv
Zoologist of the year award 2016.....	xx
Prof. Dr. A.R. Shakoori Gold Medal 2016 .....	xxii
Prof. Imtiaz Ahmad Gold Medal 2016 .....	xxiv
Gold Medals for M.Sc. and Ph.D. positions 2016 .....	xxv
<b>Research papers</b>	
USMAN, S. AND REHMAN, A. Isolation of extracellular alcohol dehydrogenase from ethanol tolerant bacteria: A potential use in biofuel production .....	1
FAIZ, A.U., ABBAS, F. LARIAB ZAHRA, L. Herpetofaunal diversity of Tolipir National Park, Azad Jammu and Kashmir, Pakistan.....	13
KHAN, I., The secrets of human heart, its condition and influence on speech and actions .....	23
AYAZ, M.M., KHAN, M.J., AZIZ, M., AHMAD, M., AKHTER, M.S., KHOSA, M.A. AND KHAN, A.A. Evaluation of single slide fecal samples of zoo animals through microfloatation technique .....	37
<b>SOME ABSTRACTS</b> .....	43
Plenary lectures .....	44
Section I: Cell Biology, Molecular Biology, Genetics, Physiology, Toxicology .....	50
1. Biochemistry, Biotechnology .....	50
2. Cell Biology and Genetics.....	57

*Continue on page 278*

[Abstracted and indexed in Biological Abstracts, Chemical Abstracts, Zoological Records, Informational Retrieval Limited, London, and Service Central De Documentation De L'ORSTOM, Paris. Also listed in Index to Scientific and Technical Proceedings and ISI/ISTP & B Online Data Base of Institute for Scientific Information, Philadelphia, Pennsylvania, USA].

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

3. Human and Animal Diseases.....	63
4. Microbiology .....	82
5. Molecular Biology .....	87
6. Physiology .....	95
7. Toxicology .....	117
Section II: Pests and Pest Control.....	143
Section III: Entomology .....	172
Section IV: Parasitology .....	193
Section V: Fisheries, Ecology, Wildlife, Freshwater Biology and Marine Biology .....	217
1. Ecology and Environmental Pollution .....	217
2. Freshwater Biology and Fisheries.....	221
3. Marine Biology.....	241
4. Palaeontology .....	247
5. Wildlife, Diversity and Conservation .....	249
Section VI: Poster session .....	273

## CONTENTS

Acknowledgements	i
Programme	ii
Members of the Congress	xi

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*Vice-Chancellor, Chief Organizer*

**Patron:** Prof. Dr. Abdul Rasool Abbasi,  
Faculty of Natural Sciences

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Department of Zoology

**Coordinator accommodation:** Prof. Dr. Ghulam Sarwar Gachal,  
Department of Zoology

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Mrs. Samina Malik	Mrs. Nosheen Jehajo	
Dr. Syma Naz	Mrs. Naheed Shah	

## PROCEEDINGS OF THE CONGRESS

*Editor*

**Dr. A.R. Shakoori**

Composed & Designed by: Amjad Ali



## **ACKNOWLEDGMENTS**

University of Sindh, Jamshoro hosted the 36<sup>th</sup> Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, University of Sindh, Jamshoro and 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, COMSTECH, Islamabad.

**36<sup>th</sup> PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)**

**UNIVERSITY OF SINDH, JAMSHORO**

**FEBRUARY 16 – 18, 2016**

**PROGRAMME**

**TUESDAY, FEBRUARY 16, 2016**

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

**JOINT SESSION I: (Plenary Lectures)**

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

**Co-chairman:** Dr. Nasreen Memon

- Speakers: 1. **Dr. Peng Xu,**  
*Director, Centre for Applied Aquatic Genomics, Chinese Academy of Fishery Sciences, 150 Yongding Rd South, Beijing, 100141, China.*  
**Development and exploitation of genome resources and tools for common carp genetic breeding**
2. **Prof. Dr. Manzoor Hussain Soomro,**  
*President, ECO Science Foundation, PSF Building, 01-Constitution Avenue, G-5/2, Islamabad*  
**Inquiry based science education for S & T innovation led economic development and international cooperation.**



3. **Dr. Wenjun Bu,**  
*Professor and Dean, College of Life Sciences, Nankai University, Tianjin 300071, China*  
**Phylogeny and evolution of Heteroptera (Insecta: Hemiptera)**

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

## **HALL – 1**

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

#### **SESSION I**

	Chairperson:	Prof. Dr. A.R. Abbasi
	Co-chairperson:	Dr. Saba Irshad
02:15 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

#### **SESSION II**

	Chairperson:	Prof. Dr. Rubina Mushtaq
	Co-chairperson:	Dr. Farah Rauf Shakoori
05:00 PM	Paper reading	
01:30 PM	Lunch and Prayer	
06:00 PM	Prayer Break (Maghrb)	

#### **SESSION III**

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

## **HALL – 2**

### **SECTION II: PEST AND PEST CONTROL**

#### **SESSION I**

	Chairperson:	Prof. Dr. Mushtaq A. Saleem
	Co-chairperson:	Dr. Riffat Sultana
02:15 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

#### **SESSION II**

	Chairperson:	Prof. Dr. Nasreen Memon
	Co-chairperson:	Dr. Hakim Ali Sahito
05:00 PM	Paper reading	
01:30 PM	Lunch and Prayer	
06:00 PM	Prayer Break (Maghrb)	

### **SECTION III: ENTOMOLOGY**

#### **SESSION I**

	Chairperson:	Prof. Dr. Tahira Jabeen
	Co-chairperson:	Dr. Zulfiqar Ali Saqib
06:40 PM	Paper reading	
08:30 PM	Dinner	

## **HALL – 3**

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

#### **SESSION I**

	Chairperson:	Prof. Dr. Wazir Ali Baloch
	Co-chairperson:	Dr. Aneela Naz Soomro
02:15 PM	Paper reading	
04:30 PM	Tea Break and Prayer Break (Asar)	

## SESSION II

	Chairperson:	Dr. Ghulam Sarwar Gachal
	Co-chairperson:	Dr. Nadir Ali Birmani
05:00 PM	Paper reading	
01:30 PM	Lunch and Prayer	
06:00 PM	Prayer Break (Maghrb)	

## SESSION III

	Chairperson:	Prof. Dr. Ayaz Qadri
	Co-chairperson:	Dr. Syma Naz
06:40 PM	Paper reading	
08:30 PM	Dinner	

## DAY TWO: WEDNESDAY, FEBRUARY 17, 2016

### JOINT SESSION II: (Plenary Lectures)

9:00 AM

**Chairman:** Prof. Dr. A.R. Shakoori  
**Co-chairman:** Prof. Dr. Akram Shah

- Speakers: 1. **Prof. Dr. Fatimah Abang,**  
*Deputy Vice Chancellor, Academic and International,*  
*Universiti Malaysia Sarawak,*
2. **Prof. Dr. Muhammad Saeed Wagan,**  
*Department of Zoology, University of Sindh, Jamshoro*  
**Natural history of *Schizodactylus* species (Grylloptera:**  
**Gryllodea: Schizodactylidae) along River Indus**  
**Hyderabad (Sindh) Pakistan**
3. **Dr. Imanullah Khan,**  
*Nuclear Institute for Food and Agriculture (NIFA), Tarnab,*  
*Peshawar, Pakistan*  
**Secrets waiting to be explored**

- 4. Dr. Zahid Beg Mirza,**  
*H. # 229-B, St. # 4, F-10/3, Islamabad 44000*  
**Impacts of climate change on some Himalayan birds**

## HALL – 1

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

#### SESSION IV

	Chairperson:	Prof. Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. Abdul Rehman
10:30 AM	Paper reading	
11:45 PM	Tea Break	

#### SESSION V

	Chairperson:	Dr. Irfan Zia Qureshi
	Co-chairperson:	Dr. Dil Ara Bukhari
12:00 PM	Paper reading	
01:30 PM	Lunch and Prayer (Zuhar)	

#### SESSION VI

	Chairperson:	Dr. Shahid Nadeem
	Co-chairperson:	Dr. Javed Ahmed Ujjan
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer (Asar)	

#### SESSION VII

	Chairperson:	Prof. Dr. Sarwat Jahan
	Co-chairperson:	Dr. Soumble Zulfiqar
05:00 PM	Paper reading	
08:00 PM	General Body Meeting	
08:30 PM	Dinner / Cultural Night	

## **HALL – 2**

### **SECTION III: ENTOMOLOGY**

#### **SESSION II**

	Chairperson:	Prof. Dr. Imtiaz Ahmad
	Co-chairperson:	Dr. Sanjota N. Das
10:30 AM	Paper reading	
11:45 PM	Tea Break	

#### **SESSION III**

	Chairperson:	Prof. Dr. Mansoor Ali Shah
	Co-chairperson:	Dr. Barkat Bughio
12:00 PM	Paper reading	
01:30 PM	Lunch and Prayer (Zuhar)	

### **SECTION IV: PARASITOLOGY**

#### **SESSION I**

	Chairperson:	Prof. Dr. Asmatullah Khan
	Co-chairperson:	Dr. A.M. Dharejo
02:00 PM	Paper reading	
04:30 PM	Tea Break and Prayer (Asar)	

#### **SESSION II**

	Chairperson:	Prof. Dr. Syed Akram Shah
	Co-chairperson:	Dr. Abdul Manan Shaikh
05:00 PM	Paper reading	
08:00 PM	General Body Meeting	
08:30 PM	Dinner / Cultural Night	

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

	Chairperson:	Dr. Abdul Aleem Chaudhary
	Co-chairperson:	Dr. Muhammad Yusuf Shaikh
10:30 AM	Paper reading	
11:45 PM	Tea Break	

**SESSION V**

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

**DAY THREE: THURSDAY, FEBRUARY 18, 2016****JOINT SESSION III: (Plenary Lectures)**

9:00 AM

**Chairman:** Prof. Dr. M. Afzal kazmi  
**Co-chairman:** Abdul Aziz Khan

- Speakers: 1. **Muhammad Moazzam Khan,**  
*Technical Advisor, (Marine Fisheries), WWF-Pakistan, Karachi*  
**Conservation of Marine Fauna and Flora in Coastal and Offshore waters of Pakistan in wake of International Instruments-Where we stand?**
2. **Dr. Zafar Iqbal,**  
*Fish Diseases and Health Management Laboratory, Department of Zoology, University of the Punjab, Lahore*  
**Import of ornamental fishes - A potential danger to local aquatic biodiversity In Pakistan**

3. **Saeed Akhtar Baloch,**  
*Chief Conservator, Sindh Wildlife Department, Opposite PIA  
Booking Office, Molvi Tamizuddin Road, Karachi*  
**Case study of the collaborative management in Pakistan  
and its success**
4. **Mohammad Irshad,**  
*Ex. Principal Scientific Officer, NARC, Islamabad*  
**Pollinators the silent forgotten friends of humanity**
5. **Habib-ul-Hassan,**  
*Department of Zoology, University of Karachi, Karachi*  
**Evolution in Marine Crustacea**

01:00 PM	Lunch Break and Prayer (Zuhar)
02:00 PM	Concluding Ceremony
02:00 PM	Recitation
02:05 PM	Congress Report by President ZSP
02:15 PM	Award Ceremony
02:45 PM	Concluding Remarks by the Chief Guest
02:55 PM	Vote of Thanks
03:00 PM	Refreshment

## MEMBERS OF THE CONGRESS

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Soomro, R.A.

#### **FOREIGN PARTICIPANTS**

Xu, P. China  
Tali, IM. JK, India  
Pir, Z.A. JK, India

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## CITATIONS

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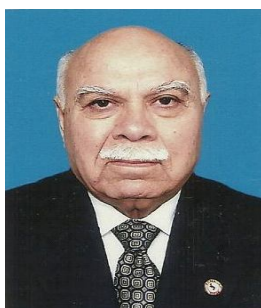
**Prof. Dr. Muhammad Saeed Wagan,**  
*Department of Zoology, University of Sindh, Jamshoro*

Dr. M. Saeed Wagan received his M.Sc degree in 1970 and Ph.D in 1976 from University of Sindh. He did his post-doctoral studies at Department of Zoology, University of Nottingham, (U.K) and at Lyman Entomological Museum and Research Laboratory, McGill University, Canada in ( 1979-1980) and (1989-1990) respectively.

During the past 44 years he has been intensively involved in research and teaching. He has published 125 research papers in national and international journals and 03 books in the field of Entomology focusing on the taxonomy, biology and ecology of Orthopteroid Insects of Pakistan. So far 14 M.Phil and 11 Ph.D degrees have been awarded under his supervision and 10 Ph. D, 05 M. Phil students are working with him at present. He successfully completed 07 research project sponsored by Pakistan Science Foundation (04), Higher Education Commission, Islamabad (HEC=01) and University of Sindh, ( 02). Beside this, he has Natural History Collection of more than Thirty thousand specimens on the order Orthoptera and related group. Dr. Wagan used his all efforts for the betterment, preservation, conservation and enhancement of Orthopteroid Insects of Pakistan throughout his career.

He has remained the Chairman, Department of Zoology, University of Sindh for three tenures. Prof. Dr. Saeed Wagan was awarded University Grants Commission merit post-doctoral scholarship in (1989-1990). He also received HEC best teacher award for the year 2004.

**RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2016**



**Mr. Zahid Baig Mirza**

*House # 229-B, Street # 4, F-10/3, Islamabad.*

Lifetime Achievement Award was awarded to Mr. Z. B. Mirza in recognition to his field research mainly on mammals and birds, particularly the wildlife of Pakistan, his published book, creative work of Natural History Museums techniques in Pakistan, awareness campaigns for the conservation of ecosystems in Pakistan, and his efforts to create conservation awareness of the officers of the civil & military armed forces and the educational institutions in the country. He is expert on painting coloured illustrations of the faunal and floral biodiversity for his books and awareness material, which are over 2,500. He remained actively involved in the pioneering international campaigns of wildlife conservation and as such he was member of WWF International team, which surveyed wildlife on the invitation of President Field Marshal Ayub Khan. His continuous efforts are well recognized for the conservation of the wildlife of Pakistan. He remained member of IUCN Environment Education Commission International and member of International Working Group on Grainivorous Birds. He is active member of Islamabad Wildlife Management Board and Chairman of its research, planning and awareness committee. He is active Board Member of Snow Leopard Foundation. He was Government of Pakistan's Consultant on Biodiversity and also consultant on the environment education capacity building in South Asia Cooperative Environment Programme. He is internationally as well as nationally decorated for his conservation and awareness efforts throughout his professional life.

**RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2016**



**Dr. Abdul Aleem Chaudhary**

*Consultant, Environment, Wildlife Biology, Ecology, Management;  
Conservation Forestry, Watershed and Range Management, Biological  
Diversity*

Dr. Abdul Aleem Chaudhary is Retired Chief Conservator of Forests, Director General Wildlife & Parks Punjab. He also served as the National Project Manager, Mountain Areas Conservancy Project, one of the major environment and conservation initiatives that have been taken in Pakistan. He has a vast experience of management of wildlife, wetlands, wildlife habitats, forests and Protected Areas in the Public Sector and facilitative work with the NGOs and the civil society.

Dr. Chaudhry planned, executed establishment and helped manage 22 Wildlife Breeding Centres/Wildlife Parks, Safari Park and two Zoos in Punjab. He was also involved in developing Management Plan of Lal Suhanra National Park. He has written at least 10 Management Plans for Protected Areas and also reviewed as many.

Dr. Chaudhry has also been teaching both at public and private universities. He taught Environmental Management at National College of Business Management & Economics University, Lahore. He has authored the Manual for Management Planning of Protected Areas for Pakistan. He has been guiding the students in producing good quality documents presenting their research findings. He has published more than 100 Research Articles. As the National Project Manager, Mountain Areas Conservancy Project he supervised the writing of management plans for conservancies and the

production of manuals for various field interventions including forestry operations, conservation issues, medicinal plants collection, processing and marketing to enhance the capacities of local/indigenous populations. He has produced more than 20 reports on Environmental Impact Assessment of different development projects including hydel projects.

He has been a Member of the Board of Governors of WWF-Pakistan, Sustainable Development Policy Institute (SDPI) Pakistan, Houbara Foundation International, Falcon Foundation International and Save Wildlife and Nature (SWAN) ---a Non Profit Company. He is also a member of IUCN Commission on Protected Areas and Species Survival Commission.

He has good communication skills that are helpful in consultations with the Government Officials, Academicians and NGOs.

**RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2016**



**Prof. Dr. Afsar Mian**

*Chairman, Bioresource Research Centre (Pakistan), 34 Bazar Road G-6/4,  
Islamabad*

Dr. Afsar Mian was born in Jassar, a village in the than District Sialkot in 1947. His father being in army, he had to face frequent shifts during his early education, starting his education from a small village school in Shinkairi to Lahore, Rawalpindi, Dak Ismail Khan, and ending up in Karachi and Quetta for the secondary school examinations. He had truly national exposure during his school education; spread all over the four provinces of Pakistan, in schools ranging from a small village “tat” Urdu medium schools to well furnished English medium city schools. This equipped him with diverse experiences and ability to work under all odds. For college education he joined Islamia college, Lahore, and M.Sc. and Ph.D. degrees from the University of the Punjab.

Immediately after the M.Sc. examination, Dr. Afsar joined Pakistan Medical Research Centre, Lahore, a University of Maryland Research Station, as a research fellow, where he got his initial research training. He produced 8 research papers during his one and half year of training; his very first scientific paper on genetically induced sterility in mosquito appeared in Science, a prestigious scientific research journal. During his regular career, he established many departments. Beginning with developing the newly coming up reproductive physiology laboratory at National Institute of Health, he was instrumental in starting a new intermediate college at Narang, a new Department of Biology at Narowal, Departments of Zoology at University of Balochistan and Bahauddin Zakariya University, and finally establishing the

Faculty of Sciences at Pir Mahre Ali Shah University of Arid Agriculture, from where he retired as Dean, Sciences, in 2007.

After his formal retirement from active service, Dr. Mian started and established yet another organization, the Bioresource Research Centre, Islamabad; the only non-profit organization of Pakistan devoted to research in management of the national bioresources. Through his consistent efforts, with the dedicated support of Dr. Fakhar-i-Abbass, Bioresource Research Centre has now a strong base for field and laboratory research in different bioresource related disciplines. BRC also has a field station at Balkasar, and an institute running M.Sc. and M.Phil. programmes in biological science. BRC is also publishing a research journal, viz., Journal of Bioresource Management in collaboration with Wright State University, USA. BRC is now well set to provide a research plat form for the biologists working in different aspects of bioresource exploration and management.

Dr. Mian is an example of a productive researcher working under all odds of newly developing institutions. He tailored his researches to limitations of the institutions in infancy and the need of the area. He produced productive research even under the environment and conditions of intermediate colleges, placed in rural areas. Starting his research in genetic control of mosquito, while at Pakistan Medical Research Centre, he has produced research in rat reproductive physiology, human population genetics, human pedigree analysis, houbara ecology, porcupine biology, fish biology, partridge ecology, wildlife ecology, and vertebrate pest biology and control. He published around 125 research papers, appearing in reputed national/and international journals, 5 books on porcupine biology and control, and some 20 biology related general articles. He has supervised Ph.D. research of 4 scholars, and a number of M.Sc. thesis research.



**RECIPIENT OF  
LIFE TIME ACHIEVEMENT AWARD 2016**



**Prof. Dr. Muhammad Arslan**

*Ex-Vice Chancellor, Quaid-i-Azam University, Islamabad  
Ex-President, Zoological Society of Pakistan*

**RECIPIENT OF  
ZOOLOGIST OF THE YEAR AWARD 2016\***



**Prof. Dr. Nasreen Memon**

*Chairperson, Department of Zoology, University of Sindh, Jamshoro.*

Dr. Nasreen Memon, Professor Meritorious, is presently working in the Department of Zoology, University of Sindh, Jamshoro. She obtained her Ph.D in 2003 from University of Karachi. She went to do National University of Singapore 2005 and then to Nottingham University, Nottingham, UK (2010). Just last year, she won the National Best Female Teacher 2014 at University Level by Federal Ministry of Education.

She supervised and produced five Ph. D students and five M. Phil students. Presently with a further nine Ph. D and fourteen M. Phil students are registered. She has also supervised more than 50 undergrad students through their research projects. Prof. Memon established the Entomology Research lab in 2004 in her department (Zoology), via two Research Projects that were funded by Higher Education Commission, Islamabad, Pakistan (HEC). In 2015, she was awarded another research project of 4.5 million rupees from HEC. She has published more than 60 research papers in well-known international journals including in UK and USA, and national journals with impact factor. Consequently, Dr. Memon has attended 62<sup>nd</sup> Annual Meeting of Entomological Society of America, Portland, USA in 2014, the Global Conference on Entomology, Thailand in 2011, the International Congress of Entomology-Durban, South Africa in 2008. She has also attended numerous meetings and seminars in UK and Singapore in 2010 and 2005 respectively.

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\*Other nominee of this award was Dr. Muhammad Razaq, Multan.

She has been Vice president (South) - Zoological Society of Pakistan since 2013, Member - Entomological Society of America; membership # 78057 since 2011, Member - Royal Entomological Society UK; membership # 7786 since 2005, Honorary member of Editorial Board of the International Journal - "Bulletin of Pure and Applied Sciences, (INDIA) since 2004 and Fellow - Pakistan Zoological Society since 2004.

Dr. Memon has given numerous lectures along with conducting workshops at different affiliated colleges on topics like the Environment, Malaria, Vector Biology, Medical Entomology and Dengue Fever (including prevention) to help increase awareness and knowledge.

**RECIPIENT OF  
PROF. A.R. SHAKOORI GOLD MEDAL 2016\***



**Dr. Muhammad Ansar**

*Associate Professor,*

*Department of Biochemistry, Quaid-i-Azam University, Islamabad.*

Dr Muhammad Ansar has received PhD degree in Biochemistry/ Molecular Biology from Quaid-I-Azam University in year 2004. In this period he initiated a research project to map and identify genes responsible for hearing loss (Deafness) in Pakistani population. These studies resulted in the mapping of three novel loci (DFNB35, DFNB38, DFNB44) and this led to the identification of disease causing genes ESRRB (DFNB35) and ADCY1 (DFNB44). Additionally, other collaborative studies resulted in the mapping of seven additional novel hearing loss loci which were later used to identify the underlying genes. Dr Ansar's group has mapped about dozen hearing loss loci and his contribution was significant in this regard which led to the identification of several new genes.

During his PhD and afterwards, he also worked on projects related with identification of genes involved in hereditary Ectodermal Dysplasias and

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\*Other applicants for this award were Dr. Furhan Iqbal, Multan, Prof. Dr. Mahmood Akhtar Kayani, Islamabad, Prof. Syed Habib Bokhari, Islamabad, Dr. Riffat Sultana, Jamshoro, Dr. Hafiz Muhammad Tahir, Sargodha, Dr. Zubia Masood, Karachi, Dr. Amanullah, Peshawar, Dr. Muhammad Usman Rashid, Lahore, Dr. Abdul Qayyum Rao, Lahore, Dr. Muhammad Ansar, Islamabad, Dr. Imran Ahmad, Peshawar, Dr. Shaukat Ali, Muzaffarabad, Dr. Muhammad Azim Khan, Peshawar, Dr. Muhammad Saqib Shahzad, Lahore, Dr. Abdul Rehman, Lahore, Dr. Asad Ali, Peshawar, Dr. Muhammad Sajjad Ansari, Gujrat

Alopecias (EJHG, 2003, 11:623 – 628; JID, 2004, 247-248). To date, Dr Ansar has published 65 research papers in International Peer-reviewed Journals with Total Impact Factor of 350 and 875 Citations according to latest JCR edition (2014), with an h-index of 15. Pakistan Council for Science and Technology (PCST) has awarded him Research Productivity Award (RPA) for several years from 2003 to 2014.

Dr. Ansar regularly attends conferences organized by Universities and Research Organizations in Pakistan and abroad. He has presented his research work in conferences organized by Pakistan Society for Biochemistry and Molecular Biology (PSBMB) and American Society of Human Genetics (ASHG). At Quaid-i-Azam University he received a research grant from Higher Education Commission (HEC) to map genes involved in Neurological Disorders prevalent in Pakistani population and the findings of this project were published in prestigious journals like Cell, American Journal of Human Genetics and Human Molecular Genetics.

Recently Dr. Ansar completed a two year postdoc from Baylor College of Medicine (BCM) at Department of Human and Molecular Genetics. While working at BCM he used genomic microarrays and whole exome sequencing to identify novel genes for achromatopsia (Hum Genet. 2015 Sep;134(9):941-50) and woolly hair (J Med Genet. 2015 Oct;52(10):676-80.). The identification of ATF6 gene in achromatopsia patients indicates the existence additional pathways responsible for color vision in humans. Dr Ansar's finding will serve as first step towards the identification of additional players and in the understanding of unfolded protein response pathways in photoreceptor cells.

**RECIPIENT OF  
PROF. IMTIAZ AHMAD GOLD MEDAL 2016\***



**Dr. Hafiz Muhammad Tahir**

*Assistant Professor, Department of Zoology, University of Sargodha, Sargodha*

Dr. Muhammad Tahir joined Department of Zoology, University of Sargodha, Sargodha in 2010, where he is currently working as Assistant Professor (Zoology). Dr. Tahir received his M.Sc and M.Phil degrees from the GC University Lahore, and Ph.D. from University of the Punjab, Pakistan in 2009. He did his post doctorate from American Museum of Natural history, New York, USA in 2013-2014. Dr. Tahir is contributing considerably both in teaching and research. His research interests include Applied Ecology, Insect Biochemistry, Molecular Systematics and Insect Pest Management. He is using scorpions and spiders as model organisms in his studies. He has published over 75 original peer reviewed articles in top journals in his field. His articles have been cited numerous times by other investigators working in this area. In recognition of research publications, he has been awarded the “Research productivity award” by Pakistan council for Science and Technology in 2011. He was awarded Young scientist Award by International Society of Arachnology in 2010. Last year (2015) he received Prof. Dr. Mirza Azhar Beg Gold Medal. He has also received several grants from Higher Education Commission, Pakistan and Punjab Agriculture & Research Board to present his research work at international conferences in Australia, USA, Germany, Spain, Malaysia, America, Switzerland and Belgium. He is serving as a Principal Investigator of three HEC funded research projects and secured handsome funding for research. He has established an advance Molecular Systematics Laboratory in his department where he is training graduate and post graduate students. He has produced one Ph.D and more than 30 M.Sc and M.Phil students. He is the reviewer of various journals of international repute.

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\*Other applicant for this award were Dr. Muhammad Razaq, Multan, Dr. Sarfraz Ali Shad, Multan and Dr. Muzammil Sattar, Faisalabad.

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

**1. Muzaffar Ahmad Gold Medal 2016**

Seventeenth Muzaffar Ahmad Gold Medal 2016 was received by Ms. Maria Hussain for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.



**Ms. Maria Hussain**

**2. Ahmed Mohiuddin Memorial Gold Medal 2016**

Twelfth Ahmed Mohiuddin Memorial Gold Medal 2016 was given to Miss Mifzala, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.



**Miss Mifzala**

**3. Muhammad Afzal Hussain Qadri Memorial Gold 2016**

Sixteenth Muhammad Afzal Hussain Qadri Memorial Gold 2016 was given to Miss. Samina for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.

**3. Afsar Mian Gold Medal 2016**

Seventh Afsar Mian Gold Medal 2016 was given to Miss. Syeda Anila Waqar who obtained first position in the M.Sc. Biology/Zoology examination of the Arid Agriculture University, Rawalpindi.



**Ms. Syeda Anila Waqar**

**4. Mujib Memorial Gold Medal 2016**

Twentieth Mujib Memorial Gold Medal 2016 was given to Miss Samina, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

**5. Prof. Dr. S.S. Akbar Memorial Gold Medal 2016**

Third Prof. Dr. S.S. Akbar Memorial Gold Medal 2016 was given to Miss Mifzala, who obtained first position in the M.Sc. Zoology examination with specialization in Entomology of the University of Sindh, Jamshoro.

**6. M.A.H. Qadri Memorial Gold Medal 2016**

Tenth Dr. M.A.H. Qadri Memorial Gold Medal 2016 was given to Dr. Syed Muhammad Hassan Mehdi Naqvi for his Ph.D. degree in Zoology specializing in the field of Parasitology from University of Karachi, Karachi.



**7. Prof. Dr. S.N.H. Naqvi Gold Medal 2016**

Tenth Prof. Dr. S.N.H. Naqvi Gold Medal 2016 was given to Dr. Muhammad Attaullah for her Ph.D. degree in Zoology specializing in the field of Toxicology from University of Karachi, Karachi.



**Dr. Muhammad Attaullah**

## ISOLATION OF EXTRACELLULAR ALCOHOL DEHYDROGENASE FROM ETHANOL TOLERANT BACTERIA: A POTENTIAL USE IN BIOFUEL PRODUCTION

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**Abstract.-** *Bacillus cereus*, *Micrococcus* sp. and *Listeria* sp. on the basis of ethanol tolerance, growth on molasses and sugarcane bagasse were selected for the present study. All bacterial isolates showed optimum growth at 37°C and at pH 7. Maximum growth was exhibited by bacterial isolates on molasses, a cheap industrial by-product. Maximum alcohol dehydrogenase activity of *Bacillus cereus*, *Micrococcus* sp. and *Listeria* sp. observed was 93%, 97% and 132%, respectively. A protein with relative molecular mass of 40 kDa was observed in all bacterial isolates after 24 h of incubation in molasses containing medium. The growth of bacterial isolates in the presence of molasses and sugarcane bagasse showed their promising potential to convert cheap raw materials into biofuel.

**Key words:** Cellulosic material, *Bacillus cereus*, *Micrococcus* sp, *Listeria* sp, Biofuel.

### INTRODUCTION

Biological fuel generation is likely to become increasingly important, especially as it provides both liquid and gaseous fuels within the next 50-100 years; particularly oil is likely to become depleted. The two main microbial fuel products currently derived from these resources are methane and ethanol (Farrell *et al.*, 2006). Biomass covers about 10% of the world's primary energy demand (Antoni *et al.*, 2007). Hence, alternative energy fuel sources that are sustainable, renewable and ecologically friendly are needed (Goldemberg, 2007).

The development of lignocellulosic-derived ethanol stems from the need for a sustainable ethanol production method. The main advantage to this method of ethanol production is the high availability of many lignocellulosic-biomass sources such as corn stover, sugarcane bagasse, cotton stalks or perennial grasses (Pandey *et al.*, 2000). Direct microbial conversion (DMC) is a method of converting cellulosic biomass to ethanol in which both ethanol and all required enzymes are produced by a single microorganism (Chandel *et al.*, 2007). The most widely used sugar for ethanol fermentation is molasses, which contains

about 50 wt% of sugar and about 50 wt% of organic and inorganic compounds, including water. It is thick, dark-colored syrup produced during refinement of sugar (Lin and Tanaka, 2006).

Several reports have been published on production of ethanol fermentation by microorganisms, and several bacteria, yeasts, and fungi have been reportedly used for the production of ethanol (Lin and Tanaka, 2006; Jia *et al.*, 2010). In addition to native ethanol producers such as *S. cerevisiae* or *Zymomonas mobilis*, several organisms capable of degrading most of the sugars present in lignocellulosic biomass have been reported; *Clostridium sporogenes*, *Clostridium indoli* (pathogenic), *Clostridium sphenoides*, *Clostridium sordelli* (pathogenic), *Zymomonas mobilis* (syn. *Anaerobica*) *Zymomonas mobilis* subsp. *Pomaceas*, *Spirochaeta aurantia*, *Spirochaeta stenostrepta*, *Spirochaeta litoralis* and *Erwinia amylovora* (Miyamoto, 1997), *Escherichia coli* KO11 (Dien *et al.*, 2003; Matthew *et al.*, 2005), *Escherichia coli* LY01 (Dien *et al.*, 2003), *Leuconostoc mesenteroides* (Miyamoto, 1997), *Streptococcus lactis* (Miyamoto, 1997), *Klebsiella oxytoca* (Matthew *et al.*, 2005), *Klebsiella aerogenes* (Ingram *et al.*, 1998).

The ethanologenic pathway in *Zymomonas mobilis*, like that of *Saccharomyces cerevisiae*, consists of two essential enzymes, pyruvate decarboxylase and alcohol dehydrogenase (ADH) (Causey *et al.*, 2004). Alcohol dehydrogenases (ADH's) are a group of oxidoreductases that occur in many organisms and are involved in the conversion between alcohols and aldehydes or ketones (Ismail *et al.*, 1993). Zinc-containing ADH's are almost always found in bacteria, mammals, plants, and in fungi (Liu *et al.*, 2000). ADH's display a wide variety of substrate specificities including molasses, starches etc.

In spite of their excellent catalytic properties, enzyme properties have to be usually improved before their implementation at industrial scale (where many cycles of high yield processes are desired). Soluble enzymes can be immobilized so that they are reusable for long times in industrial reactors. Mostly, important enzyme properties like stability, inhibition by reaction products and tolerance to alcohol have to be improved by genetic engineering (Mateo *et al.*, 2007). However extracellular ADH's can provide a simpler solution to such problems.

The present study deals with the isolation of ethanol tolerant bacteria from local environment, characterization and screening for their significant growth on cheap raw materials to produce biofuel.

## MATERIALS AND METHODS

### *Sample collection*

Soil samples were collected in sterile containers from the vicinity of different fruit shops. Temperature and pH of the soil samples were noted at the time of collection.

### *Screening of ethanol tolerant bacteria*

Selective isolation of bacteria from these samples was performed by diluting the soil samples three fold in 9% saline water. The dilutions were plated on L-agar plates and the four representative colonies were purified from each plate after incubation at 37°C for 24 h. Ethanol tolerance was checked by inoculating the bacterial isolates in 5 ml culture broth containing 5%, 10% and 15% ethanol. Growth was observed by turbidity and by taking optical density at 600 nm with spectrophotometer after incubation at 37°C for 24 h. Eight isolates designated as E1, E3, E5, E6, E7, E8, E15 and E19 were selected for further study based on their ability to tolerate 15% ethanol.

### *Growth on 1% sugarcane bagasse*

The growth of bacterial isolates on 1% sugarcane bagasse (0.5 gram dissolved in 50 ml of distilled water) containing minimal salt medium was also checked for their ability to use a cheap carbon source. Bacterial growth was determined by taking optical density at 600 nm after incubation at 37°C for 24 h.

### *Physical and biochemical characterization*

The isolates were Gram stained. For biochemical characterization the isolates were tested for catalase activity, motility, oxidase activity, nitrate reduction, and hydrolysis of casein according to Benson (1994). Some specific tests were also performed for further characterization of the isolates up to species level such as Polymyxin pyruvate egg yolk manitol bromothymol blue agar (PEMBA), blood agar, xylose lysine desoxycholate (XLD) agar and Thiosulfate-citrate-bile salts-sucrose agar (TCBS). The procedures of these biochemical tests were taken from Benson (1994).

### *Growth conditions*

The optimization of temperature was illustrated by inoculating 5ml L-

Broth in triplicate and incubating these at four different temperatures *i.e.*, 20°C, 30°C, 37°C and 42°C, while for the optimization of pH, L-broth tubes were adjusted at pH 3, 5, 7 and 9, respectively. The turbidity was observed by comparing their O.D at 600 nm after incubation at 37°C after 24 h.

#### *Effect of carbon sources on bacterial growth*

Effect of molasses and sugarcane bagasse (1% each) was determined in acetate minimal medium. For each bacterial isolate 100 ml medium was taken in one set consisting of 3 flasks (one for molasses; one for sugarcane bagasse; one for LB medium), autoclaved and then inoculated with 50 µl of the freshly prepared inoculums. The cultures were incubated at 37°C in an incubator shaker at 150 rpm. An aliquot of 3 ml culture was taken at regular intervals (0, 4, 8, 12, 16, 20, and 24 h) to measure absorbance at 600 nm. Growth in the presence of molasses and sugarcane bagasse in term of absorbance was compared with growth in LB medium of the respective isolate.

#### *Enzyme assay*

The enzyme assay was performed by harvesting the cells previously cultivated for 24 h by centrifugation at 6000 x g for 10 min and the supernatant was used as culture soluble protein. The culture soluble protein was precipitated by using 60% ammonium sulphate and was used for enzyme assay. Alcohol dehydrogenase activity was assayed by measuring the reduction of 2, 6-dichlorophenolindophenol (DCPIP) spectrophotometrically. The reaction mixture contained 0.5 ml of 0.1 mM DCPIP (0.003g DCPIP in 100 ml water), 0.5 ml of 100 mM Tris-HCl buffer (pH 7.5), 0.5 ml of substrate (1% acetaldehyde) and 0.5 ml of enzyme solution in a total volume of 2 ml. The reaction was started and optical density was taken at 600 nm after 5 min. Relative percentage reduction of acetaldehyde by the enzyme was determined.

#### *SDS-PAGE analysis of alcohol dehydrogenase*

The bacterial cultures supernatants were harvested by centrifugation at 6000 x g for 10 min at 4°C and concentrated by addition of solid ammonium sulfate (60%). The precipitate was harvested by centrifuged at 6000 x g for 10 min and dissolved in 2-3 ml of distilled water. The solution was again centrifuged at 6000 x g for 10 min and supernatant was analyzed by sodium dodecyl sulfate (SDS)-Polyacrylamide gel electrophoresis (PAGE) as described

by Laemmli (1970). The protein concentration was determined by Bradford assay using bovine serum albumin (BSA) as a standard.

## RESULTS

### *Screening of ethanol tolerant bacteria from soil samples*

Physicochemical parameters (temperature and pH) of the soil samples were at the time of sample collection. The temperature ranged between 37-42°C and pH ranged 7-8. Morphologically, nineteen different colonies observed after 24 h of incubation at 37°C. On the basis of ethanol tolerance (5%, 10% and 15%), eight bacterial isolates, E1, E3, E5, E6, E7, E8, E15 and E19, were selected. These bacterial isolates were also checked in 1% and 5% molasses. Maximum turbidity was exhibited by E6 and E7. After this, the bacterial isolates were also checked inoculated in 1% sugarcane bagasse at 37°C for 24 h. Out of 8, three bacterial isolates, E3, E6 and E7 were selected on the basis of ethanol tolerance, growth on molasses and sugarcane bagasse for further work.

### *Identification*

On the basis of biochemical and some specific tests it was confirmed that E3 was *Bacillus cereus* while E6 and E7 were found to be *Micrococcus* sp. and *Listeria* sp., respectively. No positive results were obtained with XLD agar and thiosulfate-citrate-bile salts-sucrose agar.

### *Optimum growth conditions*

*B. cereus*, *Micrococcus* sp. and *Listeria* sp. showed optimum growth at 37°C and at pH 7. In the present study, isolates E3, E6 and E7 preferred molasses over other sugar sources. Bacterial growth curves were determined in LB, molasses and sugarcane bagasse. In case of LB medium the lag phase of about 4 h was found while in molasses and sugarcane bagasse it was about 6 h of duration. E6 cells took some time to utilize these complex carbohydrates when no other carbon source except molasses and sugarcane bagasses cellulose was provided in the culture medium. The lag phase of the bacterial isolates was extended in sugarcane bagasse as compared to growth in LB and molasses media. This metabolic shifting to utilize cellulose usually slowed down the growth rate of the bacterial cells (Fig. 1).

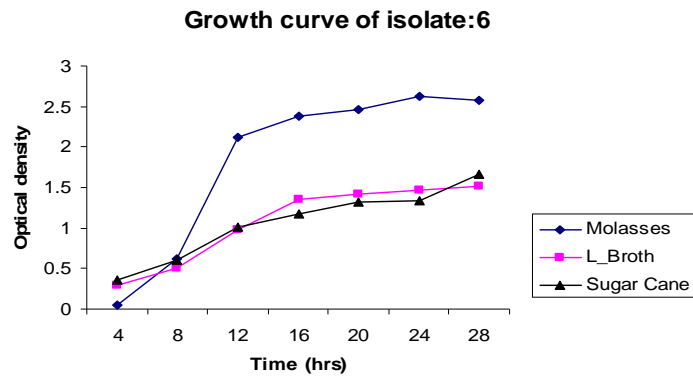
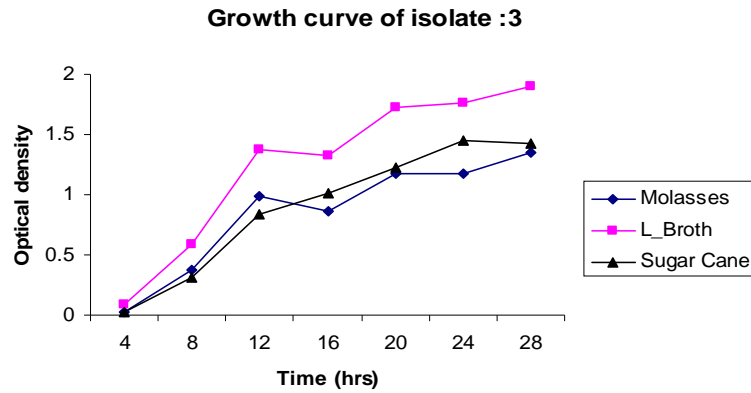
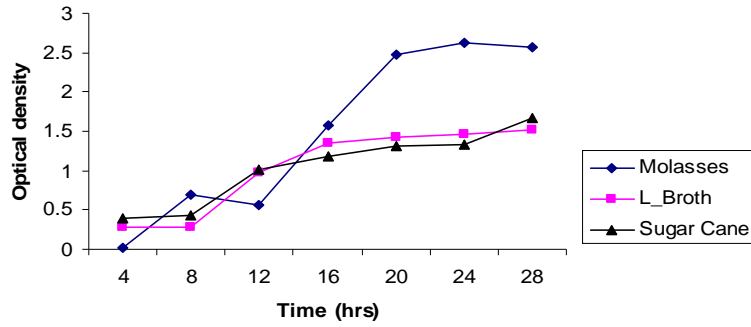


Fig. 1. Growth curves of bacterial isolates (E3, E6 and E7) in the presence of L. broth (Control) and molasses and sugarcane bagasse (Treated) as a sole source of carbon.

### Enzyme activity

Alcohol dehydrogenase activity was accessed by the reduction of DCPIP in supernatant as well as in pellet. All bacterial isolates showed high enzyme activity in supernatant when compared with the enzyme activity in pellet of the respective isolate. Alcohol dehydrogenase activity determined in *Bacillus cereus*, *Micrococcus* sp. and *Listeria* sp. was 93%, 97% and 132%, respectively (Fig. 2).

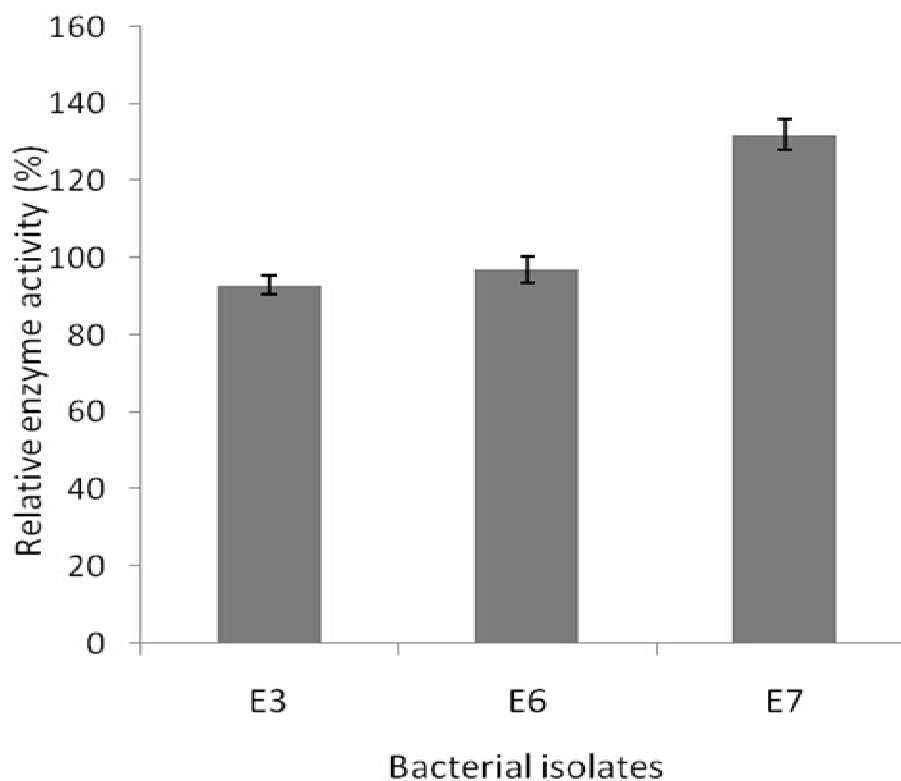


Fig. 2. Relative alcohol dehydrogenase activity (%) of bacterial isolates.

### SDS-PAGE analysis

SDS-polyacrilamide gel electrophoresis demonstrated the presence of extracellular alcohol dehydrogenase. All the isolates induced a 40 kDa protein band after 24 h of incubation in the presence of molasses (Fig. 3).



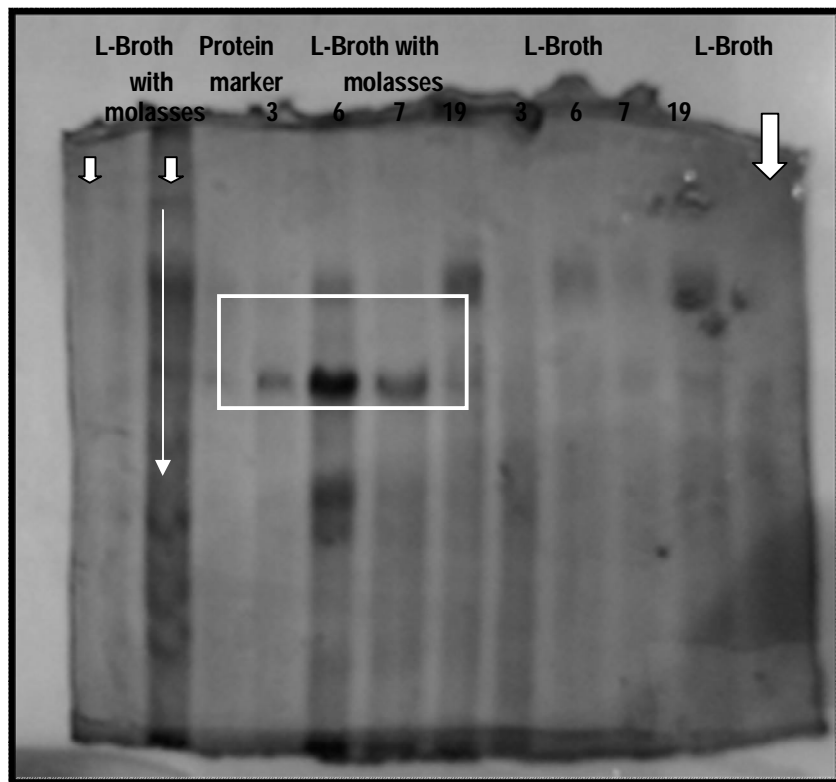


Fig. 3. Coomassie brilliant blue-stained SDS-PAGE of partially purified crude extract of bacterial isolates (E3, E6 and E7) grown in the absence and presence of molasses.

## DISCUSSION

Biofuel provides sufficient environmental benefits to merit commercial production. Transportation biofuels such as synfuel hydrocarbons or cellulosic ethanol, if produced from low-input biomass grown on agriculturally marginal land or from by-products such as molasses, could give much greater supplies and environmental advantages than food-based biofuels (Hill *et al.*, 2006; Tran *et al.*, 2010). The bacterium *Z. mobilis* also efficiently produces ethanol from glucose and tolerates up to 12% w/v ethanol (Dien *et al.*, 2003). In the present investigation, *B. cereus*, *Micrococcus* sp. and *Listeria* sp. were grown at 5%, 10% and 15% ethanol. Usually the bacteria are resistant up till 10-12%, so the

isolation of bacteria that can tolerate above 15% ethanol is expedient.

The ethanologenic bacteria have showed equal capability for industrial exploitation as *Sacchromyces cerevisiae*. Fermentation results of *Escherichia coli*, *Klebsiella oxytoca* and *Zymomonas mobilis* have been most acknowledgeable (Gunasekaran and Raj, 2006; Wang *et al.*, 2008). Gram-positive bacteria such as *Paenibacillus*, *Clostridium thermocellum* (Sveinsdottir *et al.*, 2009) and *Bacillus Subtilis* (Tran *et al.*, 2010) and thermophilic bacteria (Sveinsdottir *et al.*, 2009) have been reported for ethanol fermentation. Careful investigation has shown that limitation of carbon and major nutrients like nitrogen is responsible to some extent for the low microbial growth when fermentation is performed at high temperature or increased osmotic pressure (Gibson *et al.*, 2007).

High ethanol yields can be obtained with the conversion of sugar rich feedstocks including sugarcane bagasse, sugar beet and various fruits to alcohol (Isarankura-Na-Ayudhya *et al.*, 2007). Beet molasses and fine sugarcane directly be converted to ethanol. Molasses are a notorious by- product of the sugar cane industry (Ma *et al.*, 2008). Almost all bioethanol utilized for fuel is produced by fermentation of corn glucose in the US or sucrose in Brazil (Lin and Tanaka, 2005), but current technology for fuel ethanol fermentation can be used by any country with a effectual agronomic-based economy. All bacterial isolates, *B. cereus*, *Micrococcus* sp. and *Listeria* sp., showed maximum growth in 10 and 15% molasses, a cheap source of raw material and its employment is favorable in laboratory and commercial preparations. *B. cereus* and *Micrococcus* sp. also showed good growth in L-broth and sugarcane bagasse respectively.

Obtaining a high ethanol yield means using strains that produce ethanol with few side products, and metabolize all major sugars. Average ethanol productivity is calculated based on the final ethanol concentration and fermentation time. But of all the most important is the presence of an extracellular alcohol dehydrogenase. The concentration of alcohol dehydrogenase serves to decrease NADH levels; this finding implies that the *adh* gene is regulated by the concentration of reduced NAD (Goodlove *et al.*, 1989). Reduced NAD is in turn produced by cells grown in increased amount of sugar concentration, which means cells grown in presence of molasses should produce more alcohol dehydrogenase (Ismail *et al.*, 1993).

The presence of extracellular dehydrogenase protein was checked through

SDS-PAGE analysis and a 40 kDa protein band was observed in all bacterial isolates after 24 h of incubation in molasses containing medium. The presence of metabolic by-products is not convenient which might also suggest that the ethanol production is usually significant till 48 hours. End products of fermentation are relatively non-toxic under normal conditions in which they are primarily produced but usually become extremely toxic when accumulated in large amounts (Kascheuer *et al.*, 2006).

Bioethanol is naturally produced from pyruvate in yeast, bacteria and various other microorganisms as a result of alcoholic fermentation (You *et al.*, 2003; Kascheuer *et al.*, 2006). The ethanol fermenting pathway in bacteria, same like that of *Saccharomyces cerevisiae*, consists of two essential enzymes, pyruvate decarboxylase and alcohol dehydrogenase. Presense of extracellular or intracellular alcohol dehydrogenase can be determined by a simple alcohol dehydrogenase reduction assay (Vangnai *et al.*, 2002). NADH can reduce DCPIP; the supernatant activity is probably due to one or more soluble extracellular NAD-linked alcohol dehydrogenases (Miramar *et al.*, 2001).

Extracellular Alcohol dehydrogenase producing bacteria have a considerable potential as compared to intracellular alcohol dehydrogenase producing bacteria. These bacteria are able to tolerant ethanol up to 15% and can utilize molasses and sugarcane bagasse, cheap industrial by-products, for biofuel production.

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## **HERPETOFAUNAL DIVERSITY OF TOLIPIR NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN**

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**Abstract.-** The herpetofaunal diversity of the Tolipir National Park (TNP) provides base line data about the species composition and community structure. The herpetofauna was surveyed in summer, 2013. I recorded 4 amphibian species and 12 reptile species by visual encounter and pitfall trapping. Various diversity indices, *i.e.* Simpson Index (0.13), Shannon Winner Index (2.80), Berger-Parker Dominance Index (0.12), Margalef's Index (2.9), Menhinick Index (0.85), Buzas and Gibson's Index (0.86) were calculated to quantify the herpetofaunal diversity. The study provides base line data for future studies and comparisons of the herpetofauna of other national parks to develop a preliminary scheme for the conservation of the herpetofaunal diversity.

**Key words:** Tolipir National Park, reptile, amphibian, diversity, conservation, visual encounter survey.

## **INTRODUCTION**

Ecosystems provide goods and services, generated by the processes and interactions of biotic and abiotic components that improve human well being (Wenny *et al.*, 2011). Biotic components of a ecosystem regulate a number of maintenance processes and are responsible for the integrity and functionality of the ecosystem (Costanza and Daly, 1992; Collins and Crump, 2009). Among different biotic components, reptiles provide provisioning, regulatory, and supporting services of ecosystem. The regulatory services of reptiles are related to direct interactions between plants and animals, such as herbivory, pollination and seed dispersal (TEEB, 2010). The reptiles are valuable bio-indicators of environmental health due to philopatric to specific breeding, foraging, and overwintering habitats connected by habitats suitable for migration (Turner, 1957; Bauerle *et al.*, 1975; Duellman and Trueb, 1986; Weygoldt, 1989; Wake, 1991; Olson, 1992). Reptile provides the ideal study system to illustrate the biological and evolutionary processes underlying speciation (Raxworthy *et al.*, 2008).

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Reptile occupies a great variety of habitats: lagoons, creeks, rivers, canyons, undergrowth and tall tree habitats in forests and different jungles, as well as deserts. Some species are strictly aquatic; some specialize in terrestrial life or inhabit trees (Schlaepfer *et al.*, 2005; Wells, 2007). Reptiles are least studied among vertebrates and not frequently studied with general interest than other fauna (Bonnet *et al.*, 2002).

Some reptiles have small distributional ranges and narrow niche requirements, makes reptiles susceptible to anthropogenic threats (Anderson and Marcus, 1992). Human land use has largely negative impacts on the environment and causes habitat fragmentation isolated areas of the natural habitat, and increases habitat edges which causes significant losses among reptiles (Murcia, 1995; McGarigal and Cushman, 2002; Fahrig, 2003; Johnson, 2001; Radeloff *et al.*, 2005).

The present study is focused on the distribution and abundance of reptiles in Tolipir National Park with the objective of establishing a general overview of their position in ecosystems and conservation. No previous study is available and the herpeto fauna is not often taken into account as a conservation objective. The study of minor vertebrates is essential in management plans for any particular region for overall conservation of biodiversity at local, as well as landscape levels (Pawar *et al.*, 2007; Urbina-Cardona, 2008).

## MATERIALS AND METHODS

We conducted surveys in Tolipir National Park (TNP) between 1 April 2013 and 12 September 2013. The boundary and ravine was marked with GPS and was mapped by using Google Arce View 3.3. The total area under TNP, as determined by satellite mapping is 34.44 km<sup>2</sup> (3,444 ha = 8,150.3 acres). Since the national park is extensively hilly, a discrepancy in the area calculated through the two methods, *i.e.*, satellite mapping and ground mapping, is expected (satellite calculates area some 67.37% of the ground area). The satellite-based perimeter of the TNP has been calculated at 32.79 km. The on-the-ground perimeter, considering the hilly topography, is expected to be around 54.22 km (around 55 km).

Reptiles and amphibians were sampled via active, diurnal searches following the visual encounter survey (VES) method (Heyer *et al.*, 1994). The searches were conducted on sunny days (09:00-14:00 h) and clear evenings

(18:00 to 20:00 h). Diurnal searches consisted of walking slowly through the quadrat, thorough examination of sunny patches of habitat, slow walking through the quadrat, gentle raking through leaf litter, turning over logs boulders and rock cervices. When reptiles or amphibians were located, the following data were collected: identification, number of individuals, altitude, vegetation and microhabitat types were recorded. The encountered animals were photographed and/or preserved in formalin 70% aqueous solution for identification. The data were pooled for species richness and diversity index. Identification of photographed and preserved animals was done via Khan *et al.* (2006).

#### *Measurement of diversity*

##### *Simpson's diversity Index*

It was calculated by  $D = \sum n(n - 1) / N(N - 1)$

where N = the total number of organisms of all species and n = the total number of organisms of a particular species from which Simpson's Diversity Index,  $1 - D$ , is found (Simpson, 1949).

##### *Shannon – Wiener diversity Index*

The diversity index was calculated by using the Shannon – Wiener diversity index (1963).

$$\text{Diversity index} = H = - \sum P_i \ln P_i$$

Where  $P_i = S / N$ , S = number of individuals of one species N = total number of all individuals in the sample,  $\ln$  = logarithm to base e.

##### *Berger-Parker Dominance Index*

$$d = N_{\max} / N$$

Where  $N_{\max}$  is the number of individuals in the most abundant species, and N is the total number of individuals in the sample (Berger, 1970).

##### *Margalef's Index*

It is used as a simple measure of species richness (Margalef, 1958).



Margalef's index =  $(S - 1) / \ln N$  S = total number of species

N is total number of individuals in the sample; ln is natural logarithm.

*Menhinick Index*

$$D = s / \sqrt{N}$$

Where s equals the number of different species represented in sample, and N equals the total number of individual organisms in sample (Menhinick Index 1964).

## RESULTS

We recorded four species of amphibians belonging to two families Bufonidae (hazara toad, Maidani gauk, Asian common toad) , Family Ranidae (Kashmir nadi maindak),

We recorded twelve species of reptiles belonging to seven families - Scincidae (*Eurylepis taeniolatus*), Elapidae (*Bungarus caeruleus*, *Naja oxiana*), Colubridae (*Boiga trigonata*, *Psammophis leithii leithii*), Gekkonidae (*Hemidactylus brookii* ) Eublepharidae (*Eublepharis macularius*), Agamidae (*Laudakia tuberculata*, *Laudakia agrorensis*), Typhlopidae (*Typhlops ductuliformes*), Trionychidae (*Lissemys punctata andersoni* ) (Table II).

TABLE I.- LIST OF AMPHIBIAN SPECIES RECORDED FROM TOLIPR NATIONAL PARK (TNP).

Local name	Common name	Scientific name	Sightings	Status
<b>Family Bufonidae</b>				
Hazara ghauk	Hazara toad	<i>Bufo melanostictus hazarensis</i>	30	LC
Indus toad	Maidani gauk	<i>Bufo stomaticus</i>	23	LC
	Asian common toad	<i>Duttaphrynus melanostictus</i>	20	LC
<b>Family Ranidae</b>				
	Kashmir nadi maindak	<i>Allopaa barmoachensis</i>	25	DD

LC, least concern; DD, data deficient.

TABLE II.- LIST OF REPTILE SPECIES RECORDED FROM TOLIPIR NATIONAL PARK (TNP).

Common name	Scientific names	Sighting	Status
<b>Family Elapidae</b>			
Common krait	<i>Bungarus caeruleus</i>	20	NE
Brown cobra	<i>Naja oxiana</i>	10	CE
<b>Family Colubridae</b>			
Common cat snake	<i>Bioga trigonata</i>	13	NE
Kashmir koluber saamp	<i>Platyceph rhodorachis kashmirensis</i>	14	DD
<b>Family Typhlopidae</b>			
Slender blind snake	<i>Typhlops ductuliformes</i>	14	DD
<b>Family Trionychidae</b>			
Moonji kunchwa	<i>Lissemys punctata andersoni</i>	16	LRLC
<b>Family Agamidae</b>			
Neela kirla	<i>Laudakia tuberculata</i>	31	LRLC
Agrore wadi kirla	<i>Laudakia agrorensis</i>	59	NE
<b>Family Scincidae</b>			
Common mole skink	<i>Eurylepis taeniolatus</i>	49	DD
<b>Family Gekkonidae</b>			
Barani chipkali	<i>Hemidactylus brookii</i>	31	LRLC
<b>Family Eublepharidae</b>			
Korrh kirli	<i>Eublepharis macularius</i>	60	LRLC

**2010 IUCN Red List Category** (<http://www.iucnredlist.org/apps/redlist/details/>)

CE, Critically Endangered; DD, Data Deficient; LC, Least Concern; LRLC, Lower Risk-least concern; NE, Not evaluated.

TABLE III.- DIVERSITY INDEX OF REPTILE IN TOLIPIR NATIONAL PARK (TNP)

Diversity index	Index Range
Simpson Index	0.13
Shannon Index	2.80
Berger-Parker Dominance Index	0.121
Margalef Richness Index	2.9
Menhinick Index	0.85
Buzas and Gibson's Index	0.86

## DISCUSSION

Two amphibian families occur at Tolipir National Park (TNP) and management of the amphibian biodiversity within the park should focus on ensuring that anthropogenic threats on water quality and abundance is minimized as indicated by flow of ravine water (Fig. 1).

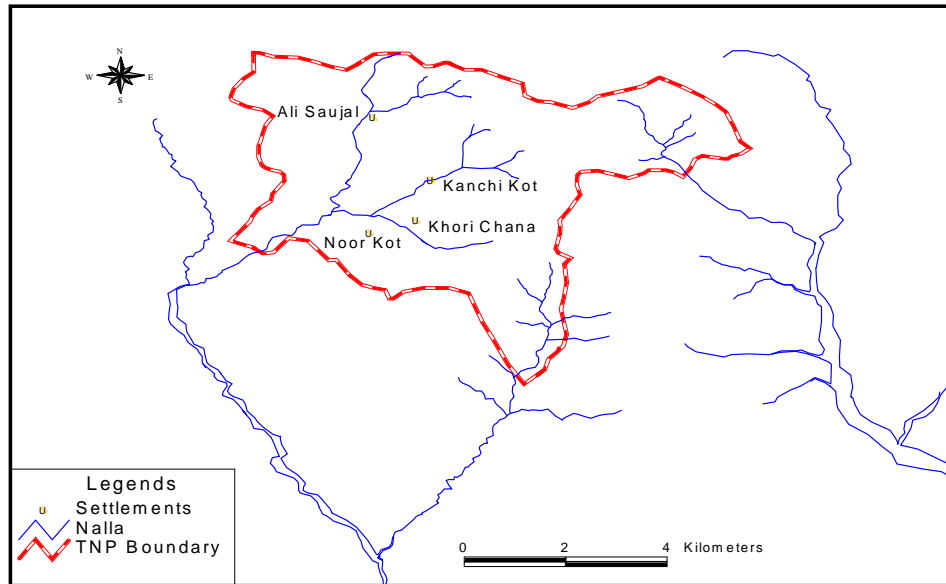


Fig. 1. Positions of the different ravines in Villages (source: Arc View 3.3 and Google Earth Pro 4.2).

Amphibian and reptile diversity in Tolipir National Park (TNP) is less diverse than Margalla Hills National Park, Pakistan which has nine amphibian species and 32 species of reptiles (Masroor 2011). The species richness of Tolipir National (TNP) is comparable to Khunjrab Nation Park (Qureshi2011) but Pir Lasura National Park (PLNP), has greater diversity where 24 reptiles, 6 amphibians are found (Madeha *et al.*, 2013). Five herp species of (5 families) were recorded Ayubia National Park (Shafique *et al.*, 2012).

The value of the Simpson Index is (0.13) which represents lower diversity because this index range 0-1, 0 represents infinite diversity and 1, no diversity. Mature and stable communities have high diversity value (0.6 to 0.9), while the communities under stress conditions, exhibiting low diversity, usually show close

to zero value (Dash, 2003). Simpson diversity index is always higher where the community is dominated by less number of species and when the dominance is shared by large number of species (Whittaker, 1965).

Typical values of the Shannon Index are generally between 1.5 and 3.5 in most ecological studies, so indicate a good diversity and the value above 3 signifies stable environmental conditions and our Shannon Index results (2.8) indicate satisfactory diversity (Stub *et al.*, 1970; Mason, 1988).

The Margalef Index has no limit value and it shows a variation depending upon the number of species. Thus, it is used for comparison of the sites (Kocatas, 1992) and takes only one component of diversity (species richness) into consideration reflecting sensitivity to sample size and our Margalef Richness Index and Menhinick indices, are in the range 2.9 and 0.85. Margalef and Menhinick indices, may be attributed to lesser number of species and environmental degradation due to anthropogenic pressures, besides other biotic factors (Ravera, 2001). The value (0.12) of Berger-Parker Dominance indicate 12% dominance while index critical value lies in the range 0-1. The value of Buzas and Gibson's Index (0.86) which measure species evenness within an ecosystem.

Due to the lack of water management particularly during snow fall, water scarcity occurs and is the main threat to the amphibian community in Tolipir National Park. Others factors are use of pesticides in agricultural pursuits and untreated waste of sewage waste being drained into main stream beds Inbreeding and habitat destruction such as loss of forest cover are also major threats to amphibian populations. This is a base line study and still population estimation and additional possible threat factors are needed to encourage future studies.

The Moonji kunchwa (use scientific name) has an association with the aquatic environment, and exhibits a limited appearance associated with small water bodies. The species is considered a low risk and may not have conservation concerns presently, yet populations of the species in the Tolipir area must be representative of specific ecotype adapted to smaller and shallow water and lower temperatures.

Common krait and brown cobra are two venomous (plants are poisonous) species were seen quite often, yet human or livestock snake bite cases have not been reported. On the global level, the brown cobra is critically endangered, while the common krait is nearly ranked as an endangered species, demanding conservation. These species are expected to face human reaction, especially after

conservation measures and hopeful population increases. Sindhi teer maar and common cat snake, which has a nearly ranked as endangered status in the global perspective, yet these are non-venomous for human and livestock. These have been regarded as data deficient species on the global level, and the conservational importance of these species is difficult to be judged. Strong human reaction appears against even the non-venomous snakes and general awareness efforts may be required to provide protection against such a negative public reaction.

There are five species of lizards, viz., korrh kirli, common mole skink, barani chapkali, black rock agama, neela kirla, and Agrore wadi kirla, which have presented their appearance in Tolipir area. These species depend upon general vegetation and insects. These species are either data deficient (common mole skink and black rock agama) or least concerned on the global level (2010 IUCN Red List Category).

### CONCLUSION

The study provides a check list of herpetofauna of Tolipir National Park (TNP) and indicates downstream water flow. This study also invites and encourages new research on the area's herpetofauna.

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## THE SECRETS OF HUMAN HEART, ITS CONDITION AND INFLUENCE ON SPEECH AND ACTIONS

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**Abstract.-** There is a compassion connection between human beings and the heart communicates with other hearts energetically or through interactions of electromagnetic fields. Sense of devotion and deep feelings originate in the heart due to external stimuli and translate into action. It is through this connection that humanity is above most of other organisms. Functionally, the heart serves as a control room for the action and behavior. The inner conscious of mankind is activated through heart. Functionally, the heart serves as a control room and communicates to the brain in four major ways: neurologically, biochemically, biophysically and energetically. Communication along all these conduits significantly affects the brain's activity, and one's behavior. The cause and effect condition of the heart is collectively called as the heart's condition. Quran gives pivotal role to the heart for understanding, feeling, storing secrets and communicating with the brain, influencing speech, and other's hearts. The present research discusses some of these facts extracted from the Glorious Quran and explained in the terminology of modern science for the guidance of scientific community in particular and humanity in general.

**Key Words:** Heart, Heart Condition, Heart Secrets, Love, Quran, Science, Tourah, Injeel, Gospel

### INTRODUCTION

For many years scientists studied heart from the physiological side and they considered it only a blood pumping machine and no more no less, but starting from the 21<sup>st</sup> century and because of the high development in heart transplantation and artificial heart surgeries, researchers started to notice a strange and vague phenomenon in the patient's psychological status after heart transplantation (Pearsall *et al.*, 2000). The latest scientific researches noticed about phenomenon in the change in psychology of the patient who exchanged his natural heart with an artificial donated one and experienced serious psychological changes in what he loves and what he hate and also in his beliefs (Pearsal *et al.*, 2005). Only from the last thirty years, researchers started to notice the relation between brain and heart and its vital role in understanding the surrounded world

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and had found that heart can affect the brain via electrical activity and have postulated the cell base therapy and immunocompatibility of tissue transplants Anjamrooz (2015). It is also documented that the human heart generates the strongest magnetic field in the body, nearly 5,000 times stronger than that of the brain. This field creates a doughnut-shaped pattern that extends well beyond the physical body and has been measured at distances of five to eight feet from the physical heart.

It may be noticed here that as we are educated over time we come across some or many of the new findings that have been foretold centuries ago in the form of Divine revelations contained in the Holy Quran. The human heart in the Holy Quran has been assigned the most important and spiritual tasks such as love, faith, acceptance, rejection, understanding, guidance, deviation, sins, intention, purification, corruption, benevolence, aggravation, invocation, negligence, fear, anger, doubt, conflict, mercy, cruelty, regret, assurance, arrogance, jealousy, rebellion, offense, and other similar acts of society. In other words, the human heart has not only been assigned the task of pumping blood into the whole body but marked with the compassion connection between human beings for the prosperity and well being of humanity.

Functionally, the heart serves as a control room for the action and behavior. Sense of devotion and deep feelings originate in the heart which translates into action. The inner conscious of mankind is activated through heart. The present discussion highlights some of these facts in the light of modern science. It is remarkable to note here that these facts were not known to many scientists even very recently. The Holy Quran has the glory to express these scientific truths that will be verified centuries later but those terms and expressions would also avoid confusing its first readers in the 7<sup>th</sup> century.

## **METHODS**

Many of the terms mentioned in the Glorious Quran such as heart condition, secrets of the heart, affixing and memorizing by heart, feeling, devotions, cognition and recognition described here are specific terms that require scientific touch on which very few scholars have given proper attention to explain them in the terminology of Modern Science. The selected terms were discussed among groups of learned scholars and common people. Related terms and verses were searched and collected from the Glorious Quran. Repeated reading of the verses and contemplation over the literal meanings of those verses were done over time. Related information on these terms were also searched

using Google and Google scholar search engines. Efforts were made to link and explain this body of information with the revealed verses in the light of science for better understanding of scientific community. In order to remove objection of the reader on originality of revealed text, some of the core references presented here are in the original Arabic text as revealed and collected in the form of Quran .

## RESULTS AND DISCUSSION

Results of the present finding of scientifically related foretold verses contained in the Holy Quran on human heart are discussed in the light of modern science for better understanding of students of biology, medical and comparative religions. By linking all these Quranic facts to the present day discoveries, it is never meant to say that Quran is a book of science but to say that Quran does contain statements (verses) suitable to the mental level of diverse individual. Quran, the miraculous book of guidance serving humanity, has instructions for all kinds of people and does not ignore any of the mental level in the society. Moreover, it also provides examples more suitable to the mental level of its first readers in the 7<sup>th</sup> century and those of present day scientists or specialists in the field of science, biology, medicine, or any other field of science..

### *The heart as an organ for understanding and feeling*

The Noble Quran informed humanity about the heart and its ability in understanding and storing thoughts and secrets (Khan, 2014a), when people viewed understanding as only controlled solely by the brain. Quran reads as,

أَفَلَمْ يَسِيرُوا فِي الْأَرْضِ فَتَكُونَ لَهُمْ قُلُوبٌ يَعْقِلُونَ بِهَا أَوْ آذَانٌ يَسْمَعُونَ بِهَا ۚ فَإِنَّهَا لَا تَعْيَى الْأَبْصَارُ وَلَكِنْ تَعْيَى الْقُلُوبُ  
الَّتِي فِي الصُّدُورِ ۖ

“Have they not traveled through the land and have they hearts wherewith to understand and ears wherewith to hear? Verily, it is not they eyes that grow blind, but it is the hearts which are in the chest that grow blind” [Quran, 22: 46].

Quran uses very strong words for the function of heart in understanding and feeling. The role of eyes and the ears in this process of understanding as commonly observed is given secondary importance and mentioned here to be under the influence of the heart. In some other place it is recited as,

وَلَقَدْ ذَرَأْنَا لِجَهَنَّمَ كَثِيرًا مِّنَ الْجِنِّ وَالْإِنسِ ۖ لَهُمْ قُلُوبٌ لَا يَفْقَهُونَ بِهَا وَلَهُمْ أَعْيُنٌ لَا يُبْصِرُونَ بِهَا وَلَهُمْ أُذُنٌ لَا يَسْمَعُونَ بِهَا ۚ أُولَٰئِكَ كَالْأَنْعَامِ بَلْ هُمْ أَضَلُّ ۚ أُولَٰئِكَ هُمُ الْغَافِلُونَ ﴿١٧٩﴾

“And certainly We have created for hell many of the jinn and the men; they have hearts with which they do not understand, and they have eyes with which they do not see, and they have ears with which they do not hear; they are as cattle, nay, they are in worse errors; these are the heedless ones” [Quran, 7:179].

Advances in science have made striking discoveries in heart functioning other than pumping of blood throughout the body and have confirmed to the divine revelations of the noble Quran. An American Psycho-neurologist, Paul Pearsall, in his book ‘The Heart’s Code Book’ Pearsall (1999) has very recently narrated that the heart also thinks, feels, remembers, and communicates with other hearts. According to his research it is the heart that one gets happy, gets angry or fell in love with someone dearer. In his synthesis of ancient wisdom, modern science proves that the heart, not the brain, holds the secrets of life.

*The human heart as an organ for memorizing*

وَإِنَّهُ لَتَنْزِيلُ رَبِّ الْعَالَمِينَ ۚ نَزَلَ بِهِ الرُّوحُ الْأَمِينُ ۚ عَلَى قَلْبِكَ لِتَكُونَ مِنَ الْمُنذِرِينَ ۚ بِلِسَانٍ عَرَبِيٍّ مُبِينٍ ۚ وَإِنَّهُ لَكَيِّدٌ زُوِيرٌ ۚ أَوَلَمْ يَكُنْ لَهُمْ آيَةٌ أَن يَكَلِّمَهُ عُلَمَاءُ بَنِي إِسْرَءِيلَ ۚ

“Verily this is a Revelation from the Lord of the Worlds: With it came down the spirit of Faith and Truth- To thy heart, that thou mayest admonish. In the perspicuous Arabic tongue. Without doubt it is (announced) in the mystic Books of former peoples. Is it not a Sign to them that the Learned of the Children of Israel knew it (as true)? [Quran, 26: 192-197].

The above verse reveals that Quran was exposed to/ affixed on the heart of Prophet Muhammad May blessings of Allah be upon him, through Angel Gabriel. The mention of heart here is very special at this occasion. ‘All the mysteries and secrets were opened to thy heart. The role of human mind in this understanding is secondary. In another place, Quran recalls the words of Moses when he was given the Torah (Gospel) an admonition for his people. Moses felt it very difficult for him to absorb and carry this message to his people and requested Allah to expand his chest for absorption, memorization and conveying this message in a lucent manner. Quran recalls this movement in the words uttered from the tongues of Moses;

قَالَ رَبِّ اشْرَحْ لِي صَدْرِي ۖ وَيَسِّرْ لِي أَمْرِي ۖ وَاحْلُلْ عُقْدَةً مِّنْ لِّسَانِي ۖ يَفْقَهُوا قَوْلِي ۖ

“(Moses) said: "O my Lord! Expand me my chest; "Ease my task for me; "And remove the impediment from my speech, "So they may understand what I say [Quran, 20:25-28].

Similarly Quran recalls the great bounty of Allah on the last prophet Muhammad peace be upon him by expanding his chest for absorption and storing of the Divine Revelation.

أَلَمْ نَشْرَحْ لَكَ صَدْرَكَ ۖ وَوَضَعْنَا عَنْكَ وِزْرَكَ ۖ الَّذِي أَتَقَضَّ ظَهْرَكَ ۖ

“Have We not expanded thee thy chest? And removed from thee thy burden. The which did gall thy back?” [Quran, 94:1-3].

During the revelation of Quran, Muhammad peace be upon him used to hast in repeating what was recited to him by the Angle Gibrail, Muhammad was instructed by almighty Allah not to make hurry but to recite slowly and gradually.

لَا تُحَرِّكْ بِهِ لِسَانَكَ لِتَعْجَلَ بِهِ ۚ إِنَّ عَلَيْكَ جَمْعَهُ وَقُرْآنَهُ ۚ فَإِذَا قَرَأَهُ فَاتَّبِعْ قُرْآنَهُ ۚ ثُمَّ إِنَّ عَلَيْكَ بَيَانَهُ ۚ

“Do not move your tongue with it to make haste with it. Surely on Us (devolves) the collecting of it and the reciting of it. Therefore when We have recited it, follow its recitation. Again on Us (devolves) the explaining of it” [Quran, 75:16-19]

Thus recitation was given the secondary importance. The first thing was the absorption of the revelation by the heart and secondly its correct pronunciation or recitation for which training was given to the Prophet Muhammad May Allah be please with him by the angle Gabriel.

For the general people it is counted as the greatest bounty from Allah when someone heart is opened to the admonition of God Almighty Allah. This can be recalled from the verse below

فَمَنْ يُرِدِ اللَّهُ أَنْ يَهْدِيَهُ يَشْرَحْ صَدْرَهُ لِلْإِسْلَامِ ۖ

“And whomsoever Allah wills to guide, Allah opens his chest to Islam (submission)” [Quran, 6:125].

بَلْ هُوَ آيَاتٌ بَيِّنَاتٌ فِي صُدُورِ الَّذِينَ أُوتُوا الْعِلْمَ وَمَا يَجْحَدُ بِآيَاتِنَا إِلَّا الظَّالِمُونَ ﴿٤٩﴾

“Nay, these are Signs self-evident in the hearts of those endowed with knowledge: and none but the unjust reject Our Signs [Quran 29: 49].

Recently experiments have demonstrated that the signals the heart continuously sends to the brain influence the function of higher brain centers involved in perception, cognition, and emotional processing.

#### *Heart as a hormonal organ*

After extensive research, one of the early pioneers in neuro-cardiology (Armour, 1991) introduced the concept of a functional "heart brain". His work revealed that the heart has a complex intrinsic nervous system that is sufficiently sophisticated to qualify it as a "little brain" in its own right. He defines that the heart's brain is an intricate network of several types of neurons, neurotransmitters, proteins and supportive cells like those found in the brain proper. Its elaborate circuitry enables it to act independently of the cranial brain – to learn, remember, and even feel and sense. His book 'Basic and Clinical Neurocardiology' (Armour and Ardell, 2004) provides a comprehensive overview of the function of the heart's intrinsic nervous system and the role of central and peripheral autonomic neurons in the regulation of cardiac function.

Another component of the heart-brain communication system was provided by researchers studying the hormonal system. The heart was reclassified as an endocrine or hormonal gland, when in 1983 a hormone produced and released by the heart called **atrial natriuretic factor (ANF)** was isolated. This hormone exerts its effects widely: on the blood vessels themselves, on the kidneys and the adrenal glands and on a large number of regulatory regions in the brain. Dr. Armour and his students also found that the heart contains a cell type known as "**intrinsic cardiac adrenergic**" (ICA) cells. These cells are classified as "adrenergic" because they synthesize and release catecholamines (norepinephrine and dopamine), neurotransmitters once thought to be produced only by neurons in the brain and ganglia outside the heart. More recently, it was discovered that the heart also secretes oxytocin, commonly referred to as the "love" or "bonding hormone." Beyond its well-known functions in childbirth and lactation, recent evidence indicates that this hormone is also involved in cognition, tolerance, adaptation, complex sexual and maternal

behaviors as well as in the learning of social cues and the establishment of enduring pair bonds. Remarkably, concentrations of oxytocin in the heart are as high as those found in the brain.

In Plato's view, emotions were like wild horses that had to be reined in by the intellect, while Christian theology has long equated emotions with sins and temptations to be resisted by reason and willpower. Quran on the other hand goes to the depth of these hormonal secretions and changes associated with it in the human behavior and command its followers to avoid such actions that will result in the secretion of enzymes/ hormones causing depression, anxiety and ill effects on human physiology in this world and punishment in the hereafter. Quran instruct its followers in the following words;

وَدُّرُوا ظَاهِرَ الْإِثْمِ وَبَاطِنَهُ ۚ إِنَّ الَّذِينَ يَكْسِبُونَ الْإِثْمَ سَيُجْزَوْنَ بِمَا كَانُوا يَقْتَرِفُونَ ۝

"Eschew all sin, open or secret: those who earn sin, will get due recompense for their "earnings." [Quran, 6:120].

Open sins are known to everyone. These are under the control and free will of doers, but the hidden sins are those known to the doers and to Allah. Some of the hidden sins such as jealousy, hypocrisy are under the control of doers but many of the hidden sins are under the control of autonomous gland secretions after the external stimuli as dirty look or desire which entrains secretions from various glands in response to these stimuli.

قُلْ إِنَّمَا حَرَّمَ رَبِّي الْفَوَاحِشَ مَا ظَهَرَ مِنْهَا وَمَا بَطَّنَ ۖ وَالْإِثْمَ وَالْبَغْيَ بِغَيْرِ الْحَقِّ وَأَنْ تُشْرِكُوا بِاللَّهِ مَا كَرِهَ يَنْزِلُ بِهِ سُلْطَانًا وَأَنْ تَقُولُوا عَلَى اللَّهِ مَا لَا تَعْلَمُونَ ۝

"Say, "The things that my Lord hath indeed forbidden are:- shameful deeds, whether open or secret; - sins and trespasses against truth or reason; assigning of partners to Allah, for which he hath given no authority; and saying things about Allah of which ye have no knowledge" [Quran 7:33].

Communication between the heart and brain is actually a dynamic, ongoing, two-way dialogue, with each organ and external stimuli continuously affects heart functioning. Researchers (Anonymous; Kalbfleish, 2008; McCraty, 2015) have shown that the heart communicates to the brain in four major ways:: neurologically or transmission through nerve impulses, biochemically (via hormones and neurotransmitters), biophysically (through pressure waves) and

energetically (through electromagnetic field interactions). Communication along all these conduits significantly affects the brain's activity.

Quran is very explicit on this functioning of the heart and all the neurological, biochemical, and biophysical communications during life. A very short Ayah summarizes this in a very beautiful way,

وَأَنَّ رَبَّكَ لَيَعْلَمُ مَا تَكْتُمُ صُدُورُهُمْ وَمَا يُعْلِنُونَ

“And most surely your Lord knows what their hearts conceal and what they manifest” [Quran, 27:74]. Similarly in verse 69 of chapter 28

وَرَبُّكَ يَعْلَمُ مَا تَكْتُمُ صُدُورُهُمْ وَمَا يُعْلِنُونَ

“Your Lord knows all that their hearts hide or reveal” [Quran, 28:69].

The verses above clearly calls its reader attention to the inside functioning of the heart and the process of causes and its effect. Whatever the heart conceals is not known to others except Allah. The biochemical functioning of heart as activated due to an external stimulus is called the heart condition. The cause and the effect due to some external stimulus resulting in biological processes such as hormonal and neural effects are all known to Allah. By this **Allah guides all the believers to control their worries, depression, emotional fatigue and withhold their sense organs especially the eyes and the ears, from indulging in unlawful actions** (Khan, 2014b), (Quran chapter 24, verse 31). Looking in lust to a strange girl, a woman, or any other person may cause the excretion of certain hormones and result unnecessary change in one's behavior. With every look (a cause) and beat of the heart (effect), intricate messages are sent to the brain and other bodily systems. In fact, these messages are being received by every cell in our body. **Controlling the external stimuli is under the free will of a person** (Khan, 2014b) **and commanded by Allah to be guarded and will help us eliminate the mental and emotional drain caused by confusion, stress, overwhelms anxiety and frustration.**

يَعْلَمُ خَائِنَةَ الْأَعْيُنِ وَمَا تُخْفِي الصُّدُورُ ۝

“(Allah) knows of (the tricks) that deceive with the eyes, and all that the hearts conceal” [Quran, 40:19].

### *Heart as an electromagnetic organ*

The human heart is the most powerful generator of electromagnetic energy in the human body, producing the largest rhythmic electromagnetic field around the body. The heart's electrical field is about 60 times greater in amplitude than the electrical activity generated by the brain (McCraty *et al.*, 2015). This field, measured in the form of an electrocardiogram (ECG), can be detected anywhere on the surface of the body. Furthermore, the magnetic field produced by the heart is more than 5,000 times greater in strength than the field generated by the brain, and can be detected up to 10 feet beyond the physical body in all directions. Research conducted at institute of HeartMath 'IHM' (HeartMath, 2005) has suggested that the heart's field is an important carrier of information.

### *The healing effect of human hearts*

The concept of an energy exchange between individuals is central to many healing techniques. This concept has often been disputed by Western science due to the lack of a plausible mechanism to explain the nature of this energy or how it could affect or facilitate the healing process. However, it has been investigated that interactions take place between one person's heart and another's brain when two people touch or are in proximity (McCraty *et al.*, 1996). Thus the heart-brain synchronization can occur between two people when they interact and their psycho physiological coherence increases due to the subtle electromagnetic signals communicated between them (McCraty *et al.*, 1997).

In the light of above scientific discussion, it has become very simple to understand some of the verses in the Glorious Quran that focus on the healing effect of human hearts.

يَا أَيُّهَا النَّاسُ قَدْ جَاءَكُمْ مَوْعِظَةٌ مِنْ رَبِّكُمْ وَشِفَاءٌ لِمَا فِي الصُّدُورِ وَهُدًى وَرَحْمَةٌ لِّلْمُؤْمِنِينَ ﴿١٠٥﴾

“O Mankind! there hath come to you a direction from your Lord and a healing for the (diseases) in your hearts -- and for those who believe, a Guidance and a Mercy” [Qura'n 10:57].

Quran explains the healing of heart disease. A very short but good review on the healing of heart diseases has been given by Khan (2014c). Here for simplicity in relevant discussion, only the sincere emotion and positive attitudes will be highlighted.



*The electricity of touch*

Owing to what is has been explained earlier as the strong electromagnetic forces around the hearts, we simply can recall many saying of our beloved prophet Muhammad as; “..None of the of Muslims when meet and Shake Hands but they get salvation before they separate” (Abu Dawoud; Tarmedi). **Thus shaking hands increase heart brain coherence, improve love and devotion and the therefore, encouraged by Islam.** The prophet of mercy has also instructed his followers to be cautious of the heart’s functioning. He has said, **‘In the body there is a piece of flesh, when it is correct, the whole body is correct, and when it is sick (senseless), the whole body will be sick, be aware! It is the heart’.**

All these verses and beautiful words of the Noble Prophet are for the activation and generation of strong bonds and coherence between the individuals with positive attitude, the effect of which can be registered on others and multiplied many folds when they are in proximity and with sincere feelings for others. The Glorious Quran also instruct its readers and followers to withstand or digest their fury at the time of fight or any other situation and exercise restraint in tiring situations. It is this forbearing or patience advised by the Creator for the betterment of His creation for training towards reaching the posted goals. It is a virtue, which enables the individual to proceed towards worthy goals, un-deflected by adverse circumstances or repeated provocations. It allows individuals to become successful. The Quran repeatedly stresses the need for patience. Throughout the Quran, we are enjoined to remain patient. The believers are instructed over 90 times to bear perseverance. Some may be quoted here,

“..Those who patiently persevere will truly receive a reward without measure!” [Quran, 39:10]. “..and bear with patient constancy whatever betide thee; for this is firmness (of purpose) in (the conduct of) affairs [Quran, 31: 17]. *And seek (God’s) help with patience and prayer.* [Quran, 2:45], And endure patiently whatever may befall thee. [Quran, 31:17], And exhort one another to be patient. [Quran, 103:3], And heed not their annoyance, but put thy trust in God. [Quran, 33:48], “have patience. God is with those that are patient” [Quran, 8: 46]. “Nor can Goodness and Evil be equal. Repel (Evil) with what is better: then will he between whom and thee was hatred become as it were thy friend and intimate! And no one will be granted such goodness except those who exercise patience and self-restraint, none but persons of the greatest good fortune. And if (at any time) an incitement to discord is made to thee by the Evil One, seek refuge in

Allah. He is the One Who hears and knows all things” [Quran, 41: 35-36]. “.....and turn off Evil with good for such there is the final attainment of the (Eternal) Home, Gardens of perpetual bliss: they shall enter there, as well as the righteous among their fathers, their spouses, and their offspring: and angels shall enter unto them from every gate (with the salutation):"Peace unto you for that ye persevered in patience! Now how excellent is the final Home!" [Quran 13:22]. “So announce the Good News to My Servants, those who listen to the Word, and follow the best (meaning) in it: those are the ones whom Allah has guided, and those are the ones endued with understanding” [Quran 39:17].

History testifies that one, who acts on impulses and emotional reactions, invariably fails; and one who responds rationally, always succeeds. Upset by opposition, jeering or other kinds of unpleasantness, he will never reach his goals. He will simply become enmeshed in irrelevancies. People with sincere thoughts and positive attitude are promised with countless bounties from their Lord for them and their peaceful wives and children will also share their company. Even the saluting angle will receive them with peace and tranquility.

#### *Why Quran forbids from getting close to fornication (Zina)*

The role of heart as an endocrine or hormonal gland that exerts its effects widely on the blood vessels, on the kidneys, the adrenal glands and on a large number of regulatory regions in the brain. The more recent discoveries that the heart secretes oxytocin (Gutkowska *et al.*, 2000), commonly referred to as the "love" or "bonding hormone has very strong influence on the behavior and action of the actor. Quran instructs its followers to overcome the unnecessary secretion of some or many of these types of hormones and refrain from anxiety and unnecessary stress.

وَلَا تَقْرَبُوا الزَّيْنَىَٰ إِنَّهُ كَانَ فَاحِشَةً وَسَاءَ سَبِيلًا ﴿٣٢﴾

“Nor come nigh to adultery: for it is a shameful (deed) and an evil, opening the road (to other evils) [Quran 17: 32].

The words ‘getting close to’ and ‘opening road to other evils’ needs special attention regarding the direct involvement of sensory organs (eyes, ears) with heart and the train of flow of several hormones that are released following the action. This will ultimately result in the anxiety and stress of the individual involved. Thus the most Merciful instructs the followers not to get close to adultery and other shameful acts because it opens door to other evil thoughts.

*Why to face accountability of hidden secrets?*

لِلّٰهِ مَا فِي السَّمٰوٰتِ وَمَا فِي الْاَرْضِ ۚ وَاِنْ تُبْدُوْا مَا فِيْ اَنْفُسِكُمْ اَوْ تَخْفَوْا بِمَا سَبَّحْتُمْ بِهٖ اللّٰهُ ۙ فَيَغْفِرْ لِمَنْ يَّشَآءُ وَيُعَذِّبْ مَنْ يَّشَآءُ ۗ وَاللّٰهُ عَلٰى كُلِّ شَيْءٍ قَدِيْرٌ ۝

“To Allah belongeth all that is in the heavens and on earth. Whether ye show what is in your self (minds/ heart) or conceal it, Allah calleth you to account for it. He forgiveth whom He pleaseth, and punisheth whom He pleaseth, for Allah hath power over all things” [Quran, 2:284].

Clearly the Quran reminds us of the unlawful indulgence and transgression after our willingness to do the shameful act for which there is penalty. We are warned to refrain from such actions that will harm our own self and result in stress, confusion and anxiety.

وَلَا تَقْفُ مَا لَيْسَ لَكَ بِهِ عِلْمٌ ۚ اِنَّ السَّمْعَ وَالْبَصَرَ وَالْفُؤَادَ كُلُّ اُولٰٓئِكَ كَانَ عَنْهُ مَسْئُوْلًا ۝

“And pursue not that of which thou hast no knowledge; for every act of hearing, or of seeing or of (feeling in) the heart will be enquired into (on the Day of Reckoning)” [Quran, 17: 36].

**Allah guides all the believers to control their worries, depression, and emotional fatigue and withhold their sense organs especially the eyes and the ears, from indulging in unlawful actions** (Quran chapter 24, and verse 31). Looking in lust to a strange girl, women, or any other person may cause the excretion of certain hormones and result in unnecessary change in one's behavior. Paying dirty gaze to someone is under the free will of the individual and the cause or stimulus for the response or beat of the heart (effect) which is under the autonomous nervous and gland system of body/ heart. **Controlling the external stimuli is under the free will of a person and commanded by Allah to be guarded and will help us eliminate the mental fatigue and emotional drain caused by confusion, stress, overwhelms anxiety and frustration. Thus man is instructed to train his free will in the right direction and refrain from unlawful acts.**

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## EVALUATION OF SINGLE SLIDE FECAL SAMPLES OF ZOO ANIMALS THROUGH MICRO FLOATATION TECHNIQUE

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**Abstract.-** Fecal samples of zoo animals are evaluated for the detection of adult parasites, larvae, ova or the segments of the endo-parasites. All relevant techniques require a considerable amount of fecal material obtained from the animals. If, in any case, there is less amount of fecal sample question arises how it can be evaluated. To evaluate a minute amount of fecal sample as low as 0.5-1.0 gram can be examined by a newly developed technique "Micro- Floatation Technique". This technique proved very much effective in qualitative and quantitative study of ova, oocyst and eggs of internal parasites. The samples obtained from zoological garden of Dera Ghazi Khan, Punjab, Pakistan were evaluated by "Micro-floatation Technique". The fecal sample of zoo animals included Black buck (*Antelope cervicapra*), Deer (*Artiodactyl cervidae*), Cheetal (*Axis axis*), Chinkara (*Gazella gazelle*), Emerald (*Amazilia brevirostris*), Neel gay (*Boselaphus tragocamelus*), Mufflin sheep (*Ovis aries orientalis*), Wolf (*Canis lupis*), Lion (*Felidae panther leo*), Goose (*Anser indicus*), Zeebra (*Equidae burcheli*) and Emu (*Dromiceius novahollandiae*) showed a low infestation of oocysts, cryptosporidium cyst, eggs of strongyles and nematodaris.

**Key words:** Microfloatation technique, fecal samples, endo-parasites, oocysts, zoo animals

## INTRODUCTION

The importance of endo-parasites present in animals can be understand by the fact that the load of nematodes, cestodes and trematodes in the body of

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animals may affect the normal body functions, production status and health (Anonymous, 1984; FAO, 2000). The presence of egg or ova in fecal sample makes the presence of parasite inside the animal. The ova/eggs are quantified through various laboratory techniques. These techniques are used daily successfully in the entire world like direct smear, concentration method, Willis technique, centrifuge floatation method, Stoll's dilution Method and Mc Master counting technique (Soulsby, 1982).

All these techniques and methods require a considerable amount of feces obtained from the animals. If, in any case, there is less amount of fecal sample question arises how it can be evaluated. To evaluate a minute amount of fecal sample of zoo animals that can be less as 0.5-1.0 gram or the fecal material of only one slide. Either it is positive or not, can be examined by a newly developed technique called "Micro-Floatation Technique".

## MATERIALS AND METHODS

The following material and reagents were used in the technique regarding the diagnosis of endo-parasites in zoo animals or wild animals in captivity.

Floatation Solution like saturated solution of Zinc sulphate was used, along with a Petri dish, a glass dropper with glass stirrer and a Sahli's apparatus (Including a glass Hemoglobin container (02 ml), small glass pipette (20  $\mu$ l) and a plastic sucker. The other equipments can be Glass slide, McMaster counting chamber, glass cover slip, ESR stand and good quality binocular microscope. The fecal samples were obtained from various species of zoo animals including Black buck (*Antelope cervicapra*), deer (*Artiodactyl cervidae*), cheetal (*Axis axis*), Chinkara (*Gazella gazelle*), emerald (*Amazilia brevirostris*), neel gay (*Boselaphus tragocamelus*), mufflin sheep (*Ovis aries orientalis*), wolf (*Canis lupis*), lion (*Felidae panther leo*), Goose (*Anser indicus*), zebra (*Equidae burcheli*) and emu (*Dromiceius novahollandiae*) kept at zoological park Dera Ghazi Khan, Punjab, Pakistan. The fecal material was scraped from the ground and side walls of the captive room with the help of glass slide or container and shifted into a small Petri dish. Dilution of the 0.5 gm. samples was diluted by 2.0 ml of Zn SO<sub>4</sub> Floatation solution in the Petri dish. The whole diluted fecal sample was shifted into Sahli's hemoglobin container (2 ml capacity) slowly and carefully by a small glass pipette (20  $\mu$ l). Hemoglobin container was fixed in ESR stand for 30 minutes so that all the ova/eggs of nematodes could accumulate and float at the upper surface of the solution. One or two drops of diluted sample

were taken after floatation process on glass slide with a glass dropper, covered by cover slip and examined under the binocular microscope. Ova/eggs of the parasites of the zoo animals were observed after placing one or two drops of floatation fluid from hemoglobin container on McMaster counting chamber and examined under binocular microscope.

During the entire process the precautions were adopted that; The entire fecal sample should be diluted by the floatation solution, the hemoglobin container having the diluted fecal sample should be fixed straight in ESR stand for the floatation and during taking the drops of diluted fecal sample from hemoglobin container, care should be taken for the spoilage of liquid due to small in quantity. Moreover one drop of Iodine solution can be added on the slide for the examination and visualization.

## RESULTS

The fecal samples of these species of zoo animals were evaluated with the help of Micro-flotation Technique” with the following results;

This technique helped to analyze the fecal samples as a minute quantity. Only 01-02 g of fecal sample was needed for the evaluation to detect the internal parasites.

## DISCUSSION

Various techniques for fecal smear to diagnose parasitic infestation are employed daily in the diagnostic laboratories as well as in centers worldwide. This technique was developed to keep in mind the delicacy of zoo animals or wild animals in captivity or in wild. These animals are shy and most often do not like manipulation for the diagnosis purposes. As these animals are precious, exotic and rare, may harbor occult or low level infections of various life threatening and public health importance parasites or may be a cause of zootherionosis, zootithasonosis and theriotithasonosis (Ayaz, 2011 and Ravindran et al., 2011). It is important to save them as well as others from those deadly parasites and to diagnose their presence well in time even if a small quantity of infection is present or meager quantity of material is available. Most of the gold standard techniques require a considerable lump of diagnostic material but some times that is also not possible to have due to innate behavior of wild animals. This micro-flotation technique has helped to overcome this

technical and practical problem successfully. The eggs of endo-parasites, ova of various nematodes and oocysts of various protozoa (Table I) can be quantified as well as can be studied qualitatively for various diagnostic and research purposes by this innovative technique. This technique can be utilized in routine at any point or even in field. Easy to carry and conduct various coprological tests.

TABLE I.- RESULTS OF PARASITIC INFESTATION IN ANIMALS OF ZOOLOGICAL GARDEN OF DERA GHAZI KHAN, PAKISTAN.

Common name	Scientific name	Number of samples examined	Number of positive samples	Percentage	Ova/cyst detected
Black buck	<i>Antilope cervicapra</i>	5	2	40	Eimeria oocyst/ Sarcocyst/ Strongyloid
Deer	<i>Artiodactyl cervidae</i>	3	2	66	Sarcocyst
Cheetal	<i>Axis axis</i>	3	2	66	Sarcocyst
Chinkara	<i>Gazella gazelle</i>	3	1	33	Sarcocyst/ Nematodaris
Emerald	<i>Amazilia brevirostris</i>	3	1	33	Not detected
Neel gay	<i>Boselaphus tragocamelus</i>	4	1	25	Sarcocyst/ Strongyles/ Aleurostrongyle
Mufflin sheep	<i>Ovis aries orientalis</i>	1	1	100	Sarcocyst/ Eimeria oocyst
Wolf	<i>Canis lupis</i>	1	Nil	Nil	Not detected
Lion	<i>Felidae panther leo</i>	1	Nil	Nil	Not detected
Goose	<i>Anser indicus</i>	3	1	33	Eimeria oocyst/ Egg of unidentified Nematodes
Zebra	<i>Equidae burcheli</i>	1	1	100	Sarcocyst
Emu	<i>Dromiceius novahollandiae</i>	2	1	50	Microscopic Eimerian cyst

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## **Some Abstracts**

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## PLENARY LECTURES

### NATURAL HISTORY OF *SCHIZODACTYLUS* SPECIES (GRYLLOPTERA: GRYLLODEA: SCHIZODACTYLIDAE) ALONG RIVER INDUS HYDERABAD (SINDH) PAKISTAN

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The genus *Schizodactylus* Brulle represents one of the most remarkable members of the order Grylloptera and was known during a long time on a single species viz. *S. monstrosus* Drury described from the shores of the river Ganga, Bengal and is mainly found in India, Bangladesh, Pakistan, Sri Lanka and Burma. *Schizodactylus* are large robustly built, ferocious looking burrowing nocturnal insects known as the “Bherwa of Bihar” and the maize cricket, although it does not belong to the family of true crickets. At the present, taxonomy, burrowing behavior, life history and ecology of the *Schizodactylus* were investigated from this region for the first time. For the collection of *Schizodactylus*, 03 sites that includes Latifabad No.4, Hussainabad and Sahrish Nagar were selected on the left bank of River Indus, Hyderabad Sindh province. All the observations were carried out during the year 2005-2009. Collected material was sorted out in three species viz., *S. monstrosus* (Drury), *S. minor* (Ander) and *S. sindhensis* the last one described as a new species to science while female of *S. minor* (Ander) is reported for the first time. With the addition of *S. sindhensis* now the total No. of known species in this genus become eight. In addition to this, a simplified taxonomic key based on the external morphological characters for the separation of adult as well as immature of *Schizodactylus* species has been presented. It has been noted that both the adults and nymphs of *Schizodactylus* are burrow makers and inhabit self-constructed burrows during the day time. Laboratory experiments demonstrated that the species of *Schizodactylus* seems to prefer to burrow in damp, cohesive sand because dry sand would collapse on them. Burrow diameter varied with the age and size of the insect. All the burrows excavated during the present study were simple and un-branched, with open or closed opening, usually straight, descending at an oblique angle into slope. In some cases burrows would gently arc or curve to the left or right along their length, sometimes away from the centre of the ridge or toward it, presumably in response to sand moisture content. The average size and depth of the burrows recorded 35.25±8.50, 49.35±8.33 and 65.3±7.34 in *S. minor*; *S. monstrosus* and *S. sindhensis*, respectively. The burrow descend at an angle of 50-60 degree varying with the slope of the surface and the sand's angle of repose, determined by the moisture content and particle size of the sand. The intervals between the individual burrow recorded 6 to 10 cm in *Schizodactylus monstrosus*, 12 cm to 30 cm in *Schizodactylus sindhensis* and 10 to 15 cm in *S. minor*. As far as biology of *Schizodactylus* is concerned it goes through 9 nymphal instars, took 1 year to complete its developmental period. *Schizodactylus* deposit approximate 23.10±3.66 eggs at 14.05±1.85 cm depth of the soil. However, *S. minor* laid the eggs along the bottom of burrow whereas the female of *S. sindhensis* and *S. monstrosus* deposit their eggs in a pit at the bottom of burrow. The number of eggs recorded 12, 34 and 45 in *S. minor*; *S. monstrosus* and *S. sindhensis*, respectively. During the field observation it has been observed that these nocturnal insects are strongly carnivorous having limited flight ability due to this they were having several natural enemies. A part from their all obliterating activities played an important role in the local food chain that prevents certain insect population from increasing and becoming dominant in field. Besides this fact, these species are important food resource for reptile i.e. snake and wall lizards and birds such as Shaheen, *Alcedo*

chukar and *Dendrocyna bicolor*. Nevertheless, ecology of three species viz., *S. monstrosus*, *S. minor* and *S. sindhensis* have been described comparatively for the first time..A total of 21960 specimens in a 1440 sampling units were collected from the Sehrish Nagar, Hussainabad and Latifabad No.4 left bank of River Indus during 2005-2009 years. The adults and nymphs were found in the white dune sand fixed grey dunes along the shore line's with the vegetation cover represented by important plants such as *Acacia nilotica*, *Tamarix gallica* and *Tamarix dioica*, *Proscopius*, *Dilbarja sessio*, *Capparis deciduas* (Kireer), *Aristolochi bracteolate* (Khabbar) which were surrounded by *Zea mays* (maize), *Saccharum officinarum* (sugarcane), vegetables, fodder crops and grasses. During the field observation little flight of this insect was noticed. Further, the record collection of *Schizodactylus* represents the first comprehensive record of the entomological fauna of Sindh. Despite the large amounts of natural history data now available for the three species of *Schizodactylus* from Pakistan. The location of oviposition and the number of eggs laid by each species each day remains a mystery still now.

### POLLINATORS THE SILENT FORGOTTEN FRIENDS OF HUMANITY

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Pollination is an essential ecosystem service that depends on symbiosis between species, the pollinated and the pollinator. The animal products like beef, mutton, poultry, and dairy products we consume are all derived one way or another from insect-pollinated legumes such as alfalfa and clover and some other plants. More than half of the world's diet of fats and oils comes from oilseeds, coconut, cotton, oil palm, olives, peanuts, rape, soybeans, and sunflower; many of these plants are dependent upon or benefited by insect pollination. Perhaps one-third of our total diet is dependent, directly or indirectly, upon insect-pollinated plants. The value of insect pollination is not limited to cultivated crops but also on uncultivated areas where most soil holding and soil enriching plants. Of the 308,006 angiosperm species 87.5% are animal pollinated with 78% species in the temperate and 94% in the tropics. Animal mediated pollination contributes to the sexual production of over 90% species of modern angiosperms. Eighty seven crops that are 70% of the 124 main crops used directly for human consumption in the world are dependent on pollinators. The production of 84% of crop species cultivated in Europe depends directly on insect pollinators, especially bees. Biotic pollination improves the fruit and seed quality and quantity of about 70% of 1,330 tropical crops. 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) which represented 9.5% of the value of the world agricultural production used for human food in 2005. Vegetables and fruits are worth €50 billion each, edible oil crops value is €39 billion, stimulants are worth €7.0 billion, nuts are €4 billion, spices value €0.2 billion and pulses 1.0 billion. In the USA, pollination value is \$ 4.5 billion; in Brazilian export of 8 important agricultural commodities it is €7 billion 5); in East Africa it is equal to €900 million); in Uganda equal to €370 million and in Netherland it is equal to €1 billion). The economic value of insect pollinators in Himalayan region of Pakistan is 954.59 million US\$). Recently the production value of pollinated dependent crop in Pakistan was quantified to be 1.59 billion US\$. Of the total value, fruits are dominant with 0.98 billion, vegetables 0.32 billion, nuts 0.15 billion, oilseed 0.13 billion and spices 0.004 billion US \$. Production of pollinated crops is not totally dependent on pollinators. There is a certain degree of dependence. Some figures have been derived from the data

for Pakistani crops. The pollinator are essential (> 90%) for Melon, Pumpkin, Water melon; great (40< 90%) for Almond, Apple, Apricot, Cucumber, Loquat, Mango; modest (10< 40%) for Guava, Jujube, Mustard, Okra, Pomegranate, Rape seed, Sesame, Sunflower; little (0< 10%) for Chille, Papaya, Persimmon, Tomatoes. There are 61 important pollinated crops used as food in Pakistan which include 26 fruit crops, 7 oilseed, 4 pulses, 19 vegetables, 2 spices and 3 nut trees. Important crops benefited by insect pollinations in Pakistan are: almond, apple, apricot, beans, cherry, coriander, citrus, coconut, cotton, cucumber, egg plant, fig, gourd/ pumpkin, guava, litchi, mango, melons, mustard, oil palm, okra, papaya, peach, pear, peas, persimmon, pomegranate, rape seed, safflower, sesame, soybean, strawberry, sunflower, and tomato. Other crops need pollinators not for production, but for seed. These include Brussels sprouts, carrot, cauliflower, lettuce, onion, and radish. Forage crops such as alfalfa and clover are also dependent on pollinator activity.

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**IMPACTS OF CLIMATE CHANGE ON SOME HIMALAYAN BIRDS TO BE  
SUPPORTED WITH MULTIMEDIA SLIDE SHOW**

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This paper gives on the impacts of climate change, on some birds of the moist Himalaya, over the last 20 years. These include observations on White Cabbage butterflies swarms' positive role in the food chain, while ascending with the thermal currents in early summer season to higher Himalayan elevations. These thermal air currents are now faster than before, that favoured the recent highest ascent of these butterflies than previously observed. So are the observations specifically on atleast 14 bird species. The adult butterflies were observed in thousands, ascending along the southern and western aspects of the hills. These were being preyed upon by several insectivorous birds that too ascended with them to alpine elevations. Observation were made using high power good binocular at 4000 ft. 11,000 ft. during March to July for 2-3 days each month. With the decreased duration of winters, hot weather prevails in the month of March, in the plains. Himalayan birds start their migration back to their breeding zones in the mountains, where at the lower elevations broadleaf flora is in the process of floral and foliage budding. But at higher zones the snow cover still prevails and the broad leaf plants have yet not sprouted. The diversity and density of singing and courting birds is high. There is also abundance of insects visiting flowers and their larvae feeding on the soft foliage. Nectar, pollens, soft fruits and seeds and insect larvae are abundantly available for the birds and for the nestlings of those birds that breed at this elevation. By the end of June the spring conditions also prevail at the Alpine Ecozone. Various birds breeding at their preferred elevations need more insect larvae for their nestlings' faster growth. Even some grainivorous birds feed their nestlings on insect larvae. Some more bird species adapted to disperse to new favourable conditions, were recorded to have ascended to new heights where they competed with the local birds for food and breeding space. Some species of local birds at alpine zone were noted to have declined due to new ecological conditions. A hairy armed bat was mist-netted at 9,000 ft. elevation, (1500ft higher than recorded before, in early July in 2014 in Kaghan valley. These observations support indications of the modification of ecological conditions of the west Himalaya, perhaps due to climatic change.

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**EVOLUTION IN MARINE CRUSTACEA**

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Darwin gave theory of origin of species by means of natural selection or the preservation of favored Races in the struggle for life (Darwin, 1850), moreover he (1959) was convinced that natural selection has been the main but not the exclusive means of modification of evolving species. Lamarck theory of Inheritance of acquired characters indicated that the effects of use and disuse of organs could be inherited and Darwin saw these effects as source of variation on which natural selection could work. Darwin incorporated the inheritance of acquired characters into his own theory of natural selection (Williamson, 1003). Significant genetic changes can be caused by natural selection in less than ten years (Dowdeswell and Ford, 1953; Sheppard, 1953). For many tropical planktonic species which have a number of generation in a year (Hassan, 1999; Mathews, 1973). This time might be even shorter. Penaeid larvae, and euphausiids were collected from oceanic plankton by net and their various appendages are drawn to show specialization and process of perfection in each group, which clearly indicate variations on which natural selection is working.

**IMPORT OF ORNAMENTAL FISHES - A POTENTIAL DANGER TO LOCAL AQUATIC BIODIVERSITY IN PAKISTAN**

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Import of ornamental fishes into Pakistan is an emerging trade. The import bill of these fish was US\$ 169,684 (Pak. Rs. 16.968 Million) in 2013. These beautiful and attractive colour pet fish are sold as healthy fish. But are these fishes are healthy or transmitting serious pathogens into Pakistan?. This question is addressed, and presented here as an overview of diseases observed in imported ornamental fishes over a period of eight years. A total of 1155 specimens of seven ornamental fish species (*Carassius auratus* goldfish n=455, and its six varieties; shubunkin n=93, comet n=50, black moor n=30, oranda n= 30, oranda n= 30, double tail n=25, fantail n=30,) and *Poecilia sphenops* molly n=100, *Xiphophorus maculatus* platy n=115; *Xiphophorus helleri* swordtail n=50; *Poecilia reticulata* guppy n=45; *Astronotus ocellatus* tiger Oscar n=100, *Cyprinus carpio* koi carp n=22) total specimens = 1155) were examined by standard laboratory procedures. The various fish species showed mild to serious external and systemic infections. Fad coloration, fin rot, skin ulcer and lesions, distended body were the main clinical signs observed in these fishes. Parasitic diseases were caused by at least seven protozoans species (*Chilodonella* sp, *Trichodina* sp; *Ichthyobodo* sp. *Epistylis* sp. *Ichthyophthirius multifiliis*; *Piscinoodinium pillulare*; *Tetrahymena* sp.); five monogenea species (*Dactylogyrus* sp. *D. vastator*, *D. extensus*; *Gyrodactylus* sp. *G. turnbulli*); digenea (*Cryptocotyle* sp.), two nematode species (*Camallanus* sp. *Capillaria* sp.); two crustaceans species (*Argulus foliaceus*, *Lernaea cyprinacea*). Fungal infections were caused by *Aspergillus* spp. *Alternaria* sp, *Penicillium* sp. *Mucor* sp. *Blastomyces* sp. *Rhizopus* sp. *Fusarium* sp. Bacterial diseases such as; motile aeromonad septicaemia, fin rot and haemorrhagic ulcers on skin are also reported from some of these ornamental fishes. Parasitic infections were the most prevalent and serious in these fishes. Fish diseases diagnosed and their

treatments are discussed. Strict regulations on import of ornamental fish may be imposed urgently to control the transmission of serious pathogens into the country to avoid probable catastrophic situation in our aquatic biodiversity in future.

### SECRETS WAITING TO BE EXPLORED

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Quran is the last Divine Book revealed for the guidance of human being and addresses both the ignorant and learned people of society according to their mental capacity to read and contemplate over the signs of magnificent designer and creator of the universe. The Quran exhorts its reader and specifically the believers that the pursuit of exploring this universe is one of their religious duties that will guide them to the existence of One God. In the present discussion we have focused on the specific verses of the Glorious Quran that address the most learned people of human society. Some of these verses contain glimpses of established scientific signs while other will focus on the foretold facts that will be discovered in coming time. As we are educated over time, we see that there are hundreds of scientific signs still waiting for discoveries by the scientists. Some of the awaited discoveries include the reality of information stored in the body cells or DNA [Quran 6:67], the testimony of body parts on the Day of Judgment, the fulfillment of sustenance of all living beings from the Heaven [Quran 51:22, 45:4-5], the soul and its destination [Quran 83:18-21, 56:83-9], the secrets of hearts, their storage and retrieval [Quran 22:46, 100:10] on the Day of Judgment, Why are hearing, sight and even heart accountable? [Quran 17:36,]. The reality of spiritual enlightenment by Divine guidance [Quran 5: 15-16, 6: 91], and radiating faces on the Day of Judgment. [Quran 57:12-15]. The mind set of matter, particles, atoms, their capacity in learning the events and obeying One God.

### CASE STUDY OF THE COLLABORATIVE MANAGEMENT IN PAKISTAN AND ITS SUCCESS

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Under the legislations, hunting, shooting and habitat destruction is strictly prohibited in National Parks and Wildlife Sanctuaries, whereas hunting and shooting of wild animals under special permits is allowed in Game Reserves as well as Community Conservation Areas/ Private Game Reserves.

IUCN Category II applies to the most of National Parks in Pakistan

IUCN Category IV applies to the most of Wildlife Sanctuaries in Pakistan

IUCN Category VI applies to the most of Game Reserves and Community Conservation Areas/ Private Game Reserves in Pakistan.

Ziarat Juniper Forest (Balochistan Province) has recently been declared as Biosphere Reserve under the UNESCO's Man and Biosphere (MAB) Program. Earlier in 1977, Lal Suhanra National

Park (Punjab Province) was declared as Biosphere Reserve. In Pakistan Community-based Trophy Hunting of ungulates; Markhor, Urial (*Ovis vignei*) Blue Sheep (*Pseudois schaeferi*), Himalayan Ibex (*Capra sibirica*), Sindh Goat (*Capra aegagrus*) is being successfully implemented in Pakistan and is world recognized. The local communities get 80% shares of the revenue generated, which is used for conservation and socio-economic uplift. 20% of the revenue goes to government as administrative fee. In return the communities protect wildlife with a sense of ownership. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has allocated quota of 12 Markhors for Pakistan. In 2010 at the 10th Conference of Parties of the Convention on Biological Diversity in Nagoya, Japan, Markhor Award of the International Council for Game and Wildlife Conservation (CIC) was given to the Torghar Conservation Programme, Balochistan, Pakistan recognizing outstanding conservation performance through Community-based Trophy Hunting Program.

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## SECTION - I

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

#### 1. BIOCHEMISTRY, BIOTECHNOLOGY

##### ESSENTIAL AND NON-ESSENTIAL AMINO ACID COMPOSITION OF INDIAN AND CHINESE MAJOR CARPS FROM THE PERSPECTIVES OF FISH IMMUNITY AGAINST *LERNAEOSIS*

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Gradual intensification of fish culture has domino effect on incidences of various parasitic and non-parasitic diseases especially *Lernaeosis*- the most common deadly disease of carps. However, the balanced amount of amino acids in the living organisms sustain normal immune-competency and protect from the pathogenic diseases. Grass carp (*C. idellus*), silver carp (*H. molitrix*), rohu (*L. rohita*), mrigal (*C. mrigala*), thaila (*C. catla*) and common carp (*C. carpio*) were cultured in semi intensive ponds at Fish Farm Complex, University of Veterinary and Animal Sciences, Lahore. Parasitic infestation was observed in all fish species in the months of June, July and August, 2012. Infected ponds were treated with Thander (DDVP- an organophosphate) to eliminate the parasite. Triplicate samples (830 ± 316 g) of parasite free and *Lernaea* infected were collected for whole-body amino acid composition using Agilent chromatograph, reverse phase high performance liquid chromatography (HPLC). Statistical analysis revealed highest *L. cyprinacea* infestation in *C. catla* whereas, not a single parasite was recorded on *C. carpio* in the month of June and July. *C. mrigala*, *H. molitrix* and *C. idella* showed significant infestation. *L. rohita* however, has very low level of infestation. Abdomen and caudal fin were the most attractive sites for the parasitic infestation followed by the dorsal fin, head and gills, respectively. After repeated medication, parasite was completely eradicated from the ponds in the month of August, 2012. There was consistent decrease in amino acid values from *C. carpio* to *C. mrigala*, *L. rohita*, *H. molitrix*, *C. idella* and *C. catla*, respectively except the cysteine and methionine. Trend and level of significance was the same in lysine, leucine, isoleucine and threonine whereas, tryptophan was the only amino acid detected in *C. carpio*. *C. carpio* possessed the highest values for all non-essential amino acids and the lowest in *C. catla*. Values of aspartic acid and proline significantly varied among species. Significantly higher values of essential and non-essential amino acids in parasitic free fishes indicating good health; high nutrition values means strengthening of fish immune system and hence high resistance and low susceptibility to pathogens.

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### MINERAL CONTENTS IN DIET OF BLACK BUCK (ANTELOPE CERVICAPRA) COLLECTED DIFFERENT FROM CAPTIVE AND SEMI-NATURAL CONDITIONS

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Foodstuff from different captive sites Lahore Zoo, Bahawalpur Zoo, Lahore Zoo Safari and Lal Suhanra National Park Bahawalpur was collected to determine the mineral content in diet of Black Buck. Food variety was different at different sites. Animals were feeding on same food *i.e* Lucerne and parched grams at three captive sites, Lahore Zoo, Bahawalpur Zoo and Lahore Zoo Safari. In contrast to that animals in Lal Suhanra were feeding on grain, barseem (*Trifolium alexandrium*), oat (*Avena sativa*), Lucerne, Jowar (*Sorghum bicolor*) Kikar (*Prosopis juliflora*) Beri, Jandi (*Prosopis cineraria*) at regular basis during the survey durations. Foodstuff was analyzed through mineral extraction method to find out the mineral concentration in fodder and diet. All samples were analyzed under standard protocol in Veterinary Research Institute lab Lahore. Minerals such as Na, K, Ca, Mg, and P were observed in various concentrations along with the trace elements. It was noticed that there were striking differences in mineral contents of Black Buck fodder collected from all enclosures. Black bucks of Lal Suhanra were having remarkable body sheen, breeding rate, amazing physical appearance. This was due to their better diet at their enclosure. Natural behavior of these animals was seen only in animals of Lal Suhanra while all these characters were almost absent in black bucks of other sites. We concluded from the study that these threatened animals are being compromised in captive conditions. There is need to revise the adapted ration scale by management of captive sites.

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### ASSOCIATION OF CANNABIS ABUSE WITH LIFE STYLE AND DIETARY FACTORS: A CASE CONTROL STUDY

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Cannabis plant and its products consist of an enormous variety of chemicals. Cannabis contains over 300 compounds. At least 66 of these are cannabinoids. Prevalence of cannabis use in Asia is low between 1.2% and 2.5% of the population 15 to 64 (31 to 68 million people). Most commonly used drug in Pakistan consumed by 3.6% of the adult population or four million people listed as users of drug use. Hence, to see the association of cannabis abuse and lipid alterations we undertake the present study. The Cannabis is used in two forms in Pakistan, hence, to see the effect of cannabis use on their serum lipids and dietary habits, they were divided into two groups: (1) Cannabis drinkers and (2) Cannabis smokers. Age, gender and locality matched healthy controls with negative personal and family history of cannabis use were taken. The drug abuse screening

test (DAST-10) was administered. Dietary habits, BMI, age, gender, occupation, family history of cannabis abusers in comparison to controls were also assessed by a standard questionnaire especially designed for that purpose. We explored that the majority of cannabis abusers were of 16 to 30 years old as compared to controls. Among the randomly selected cannabis abuser majority were physically active as compared sedentary life style. Majority of cannabis abuser consume underground water. Drug Abuser Screening Test (DAST) revealed the majority of cannabis abuser was at the substantial level of addiction (6-8 score) and needed intensive assessment. It was explored that there was a significant positive association of light tea ( $p<0.01$ ) when strong tea was taken as reference ( $OR=1$ ). Ghutka and Naswar were significantly positively associated ( $p<0.01$ ) with cannabis smokers when no other addiction was taken as reference. Significant positive association ( $p<0.01$ ) of 2 times/day meal was found with cannabis smoking and cannabis drinking. The smoking of 11-21 No. of cigarettes /day was significantly positively associated ( $p<0.01$ ) with cannabis smoking and drinking. Cannabis smoking as well as drinking was also strongly positively associated ( $p<0.01$ ) with smoking as compared to non- smokers. Cannabis smoking was significantly positively associated ( $p<0.01$ ) with low blood pressure when normal blood pressure was taken as normal. In conclusion, cannabis abusers were positively associated with ghutka, naswar, 2 times/day meal intake, 11-21 cigarettes/day and low blood pressure.

#### **IMPORTANCE OF RAW MILK QUALITY FOR CHEESE MANUFACTURING**

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Ranking of milk production countries of Pakistan around the globe is 4<sup>th</sup>. 70% population of Pakistan lives in villages, which means that higher milk production in villages. As villages are far away from the cities, so milk is transported to cities by vehicles which are time consuming and quality affecting. Due to short shelf life of milk, it is necessary to preserve raw milk as soon as possible. For preservation, adulteration techniques are performed to increase shelf life, which affect the natural milk composition and unbalancing of SNF/Fat ratio. This adulterated milk is used for processing of different milk products. Due to increased trends of fast food consumption in Pakistan, demand for cheddar and mozzarella cheese is also mounting. But due to less availability of good quality raw milk, adulterated/ poor quality raw milk is used for cheese making. As milk proteins play an important role for cheese making, so adulterated milk will result in low quality cheese. Cheese prepared from good quality raw milk has better chemical and sensory characteristics as compared to cheese prepared from adulterated milk. Good quality milk help to make firm coagulum, better yield better textural and flavour characteristics. This will make our fast food very delicious. Adulterated milk will result in less quality cheese, loose texture and bad sensory attributes. If adulteration is done with vegetable oil, cheese will be oily and loose texture. If adulterated cheese is used in pizza making, then oil separation and bad smell can be observed. It results in loss to industry and less customer satisfaction. So, industries are trying to obtain good quality raw milk for better yield and better customer satisfaction even at higher prices. Cheese industry can be boosted in the country through the provision of good quality raw milk.

### CADMIUM RESISTANCE MECHANISM IN *ESCHERICHIA COLI* P4 AND ITS POTENTIAL USE IN WASTEWATER TREATMENT

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A cadmium resistant bacterium was isolated from industrial wastewater and identified as *Escherichia coli* (dubbed as P4) on the basis of morphological, biochemical tests and 16S rRNA ribotyping. It showed optimum growth at 30°C and pH 7. *E. coli* P4 found to resist Cd<sup>+2</sup> (10.6 mM) as well as Zn<sup>+2</sup> (4.4 mM), Pb<sup>+2</sup> (17 mM), Cu<sup>+2</sup> (3.5 mM), Cr<sup>+6</sup> (4.4 mM), As<sup>+2</sup> (10.6 mM) and Hg<sup>+2</sup> (0.53 mM). It could remove 18.8%, 37% and 56% Cd<sup>+2</sup> from aqueous medium after 48, 96 and 144 h, respectively. FTIR, SEM and EDX analysis also confirmed the biosorption of Cd<sup>+2</sup> by *E. coli* P4. However temperature and pH were found to be most critical factors in biosorption of Cd<sup>+2</sup> by *E. coli* P4. Cd<sup>+2</sup> stress altered *E. coli* P4 cell physiology analyzed by measuring glutathione (GSH) and non-protein thiol (cysteine) levels which were increased upto 130% and 48%, respectively. qRT-PCR showed alteration in the expression levels of *ftsZ*, *mutS*, *clpB*, *ef-tu* and *dnaK* genes in the presence of Cd<sup>+2</sup>. Total protein profiles of *E. coli* P4 in the absence and presence of Cd<sup>+2</sup> were compared by SDS-PAGE, which showed remarkable difference in the banding pattern. *czcB* gene, a components of *czcCBA* operon, was amplified from genomic DNA which suggested the chromosomal-borne Cd<sup>+2</sup> resistance in *E. coli* P4. Furthermore, it harbors *smtAB* gene which plays significant role in Cd<sup>+2</sup> resistance.

### EFFECT OF DIFFERENT BROODING SOURCES ON SERUM CHEMISTRY AND IMMUNE RESPONSE OF 3 COMMERCIAL BROILER STRAINS

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Present study was conducted to evaluate the effect of different heating systems using 720 day-old commercial broilers, 240 from each of the 3 strains (Ross 308, Cobb 500, Hubbard classic). All the birds were randomly distributed into 36 replicates having 20 birds each. The birds were subjected to 4 heating systems during brooding (Diesel Brooder, Floor Heating, Liquefied petroleum gas and Electricity Bulb). At day 7<sup>th</sup>, 16<sup>th</sup>, 36<sup>th</sup> and 42, four birds were randomly selected from each treatment to get 3 ml blood to determine the blood glucose, cholesterol, total protein, urea and albumin along with immune response against the Newcastle (ND) and Infectious bursal

disease (IBD). The data thus recorded were analyzed through Completely Randomized Design under factorial arrangement. The comparison of means was made using Duncan multiple range (DMR) test. Results revealed that the Ross 308 strain reared on floor heating system exhibited significantly better immune response against ND and IBD as compared to other heating system, while, serum glucose was higher in Hubbard on Liquefied petroleum gas. It can be concluded that the Ross 308 strain maintained on floor heating system(FH) exhibited improved immune response and better serum chemistry in comparison to other broiler strains maintained under different brooding systems.

**IN-SILICO ANALYSIS OF LOOP RESIDUES OF THE RECEPTOR BINDING DOMAIN OF *BACILLUS THURINGIENSIS* CRY2AC11 TOXIN**

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Genetic engineering of *Bacillus thuringiensis* Cry proteins has resulted in the synthesis of various novel toxin proteins with enhanced insecticidal activity and broader specificity spectrum. Toxin-receptor interaction is known to be the most crucial and rate limiting step for toxin activity and specificity determination but still very less information is available regarding the receptors of Cry2 proteins and the mechanism of receptor-ligand interactions of BT toxins. In the present work, detailed *in-silico* studies of the binding epitopes of *Bacillus thuringiensis* Cry2Ac11 toxin are done. To get the knowledge about the binding characteristics of each single residue in the receptor binding region, various bioinformatics tools are employed and protein modeling, sequence and structural homology analysis, alanine scanning and protein docking studies are done. Also the binding interactions of individual residues with different lepidopteran and dipteran receptors are predicted. The collective knowledge of Cry toxin interactions with its potential receptors will lead to a more critical understanding of the structural basis for receptor binding and specificity determination. This study will serve as a starting point for the design of mutagenesis experiments aimed to improve the insecticidal activity of Cry proteins.

**BIOSYNTHESIS OF 1, 4- $\alpha$ -D-GLUCAN GLUCANOHYDROLASE FROM BACTERIAL CO-CULTURE USING SOLID STATE FERMENTATION**

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The present study revealed the selection of suitable bacterial co-culture for 1, 4- $\alpha$ -D-glucanglucanohydrolase (GGH) production. 208 compatible co-cultures were screened for 1, 4- $\alpha$ -D-glucanglucanohydrolase production employing solid state fermentation. The co-culture showing highest GGH producing potential were identified both by biochemical testing and 16S rRNA

sequencing technique. The result revealed the selected co-culture consists of *Bacillus cereus* and *Bacillus thuringiensis*. Eight different fermentation media were evaluated for GGH production. Among all the tested media, M 5 containing wheat bran and potassium phosphate buffer gave maximum enzyme production. Different Physical parameters such as incubation time, moistening agents, incubation temperature, pH and inoculum size were investigated. The optimal GGH production by co-culture was obtained at 48 h, 37°C, pH 7, inoculum size of 4ml in the presence of potassium phosphate buffer as moistening agent. The influence of other variables such as carbon and nitrogen sources was also determined. 4% starch and 1% tryptone were also optimized as best carbon and nitrogen sources for the enhanced production of GGH. The novelty of this study is the exploitation of synergistic phenomena of bacterial strain by the use of co-culture technique for the enhanced production of GGH

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#### **BIOREMEDIATION OF METALS- AND SULPHATE-LOADED WASTEWATERS USING AGRO-INDUSTRIAL WASTES AS COST-EFFECTIVE GROWTH SUBSTRATES**

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We conducted a study to treat artificially prepared metals- and sulphate-loaded wastewater while employing metal-resistant dissimilatory sulphate-reducing bacteria (DSRB) as remedial agents. For the purpose, two SRB species were isolated from a wastewater channel in Pakistan, characterized phenotypically as well as genotypically and employed in the remediation of the wastewater. In order to economize the remedial process, eleven different agro-industrial wastes were employed as cost-effective growth substrates and included apple peelings, apple pomace, mango peelings, watermelon rind, composted bovine manure, fresh bovine manure, poultry litter, poultry manure, molasses, sugarcane bagasse and sawdust. The SRB species were cultivated independently on each organic waste and the overall sulphate reduction performances were examined in a 60-day trial of anaerobic incubation. The results declared apple pomace and composted bovine manure as the most efficient carbon sources / growth substrates supporting prodigious growths of *Desulfovibrio fructosovorans*-HAQ2 and *Desulfovibrio desulfuricans*-HAQ3 and thus resulted in maximum sulphate reductions up to 76 and 73%, respectively. In the second phase of the study, these bacterial species were employed using apple pomace and composted bovine manure for the precipitation of four concentrations (1, 5, 10 and 15 ppm) of three dissolved metals (Cu, Cr and Ni) independently. Both SRB species showed results in a very narrow range of fluctuations. In general, bioprecipitation was pronounced at lower concentrations (1 and 5 ppm) and get inhibited at higher concentrations (10 and 15 ppm). The order of precipitation for the subject metals appeared as Ni < Cr < Cu. The findings of this study will be helpful in developing economical and environmental friendly bioremediation process(es) addressing inexpensive treatment of metals' and sulphate rich wastes with concomitant consumption / removal of organic pollutants.

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**BIOETHANOL PRODUCTION FROM URBAN CELLULOSIC WASTE EMPLOYING  
*ALCALIGENESFAECALIS* HI-1 ISOLATED FROM GUT OF TERMITE  
*HETEROTERMES INDICOLA***

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The present study was designed to produce bioethanol from cheap urban cellulosic waste office paper by utilizing cellulolytic bacterial isolates from the gut of local termite specie *Heterotermesindicola*. Among various isolates, the highest cellulolytic bacterial isolate was selected on the basis of primary screening and secondary screening and was optimized at different physical and chemical conditions. Optimum growth and maximum glucose yield was obtained at temperature 37°C, pH 4.5, 5% (w/v) substrate concentration, 6% bacterial inoculum size, agitation at 150 rpm and in the presence of PEG and Ca<sup>2+</sup> ions. Overall 8 fold increase in glucose yield was achieved. The yield of bioethanol obtained through simultaneous saccharification and fermentation (SSF) of office paper was 5.43 mg/ml. According to 16S rRNA sequence homology, the bacterial isolate H1 was identified as *Alcaligenesfaecalis*. Bioethanol production from untreated waste office paper proved effective strategy. Bacteria having natural tendency towards cellulosic waste consumption are promising for cellulosic waste bioconversion to valuable products and can be utilized in various applications. Bioconversion of urban cellulosic waste to valuable products environmental friendly.

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## 2. CELL BIOLOGY, GENETICS

### ASSESSMENT OF GENOTOXIC POTENTIAL OF PESTICIDE MIXTURE FOR THE FRESHWATER FISH, *CYPRINUS CARPIO*

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Over the past few decades residues of pesticides reach the aquatic environment where it poses significant toxicological risks to a myriad of non target organisms, ultimately finding their way to the food chain threatening the ecological balance and biodiversity of the natural world. Therefore, this study aimed to evaluate the genotoxic effects of two commonly used pesticides, bifenthrin and chlorpyrifos mixture on commercially important freshwater fish, *Cyprinus carpio*. For this purpose 180 day old fish fingerlings were exposed to different sub-lethal concentrations viz. 33, 25, 20 and 16% of 96-hr LC<sub>50</sub> of mixture for a period of one month at constant laboratory conditions. Fish peripheral blood erythrocytes were sampled on 15 and 30 days of exposure for the assessment of DNA damage. Genotoxic effects of mixture were determined in-terms of percentage of damaged cells in 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 cells were observed significantly higher on day 30 at all sub-lethal concentrations as compared to 15 day of exposure. The DNA damage was found to be dose and time dependent with highest DNA damage observed at 33%, followed by that of 25, 20 and 16% of LC<sub>50</sub> exposure as compared to control groups. This study confirmed that the comet assay is a useful tool for assessing the genotoxic potential of water pollutants and might be appropriate as a part of environmental monitoring programs.

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### PREVALENCE OF STROMAL CORNEAL DYSTROPHIES IN PAKISTANI POPULATION

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Present retrospective, analytical study was performed between November 2014 and July 2015 on all cases seen from different cities of Punjab during ophthalmological consultation at the Layton Rahmatullah Benevolent Trust hospital, Mughal hospital, Mayo hospital and General Hospital. The aim of this research was to determine the prevalence of Stromal Corneal Dystrophies (SCDs) in Pakistani population. The techniques used for the diagnosis of Stromal Corneal Dystrophies were an examination of cornea using a slit lamp biomicroscopy, specular microscopy, topography, keratometry, orbscan and far visual acuity. 50 cases of Stromal Corneal Dystrophy were identified from Punjab, corresponding to hospital prevalence of 0.5%. The Stromal Corneal Dystrophies were studied in 20 females and 30 males. Stromal Corneal Dystrophies is predominant in age group of 30-40 years. Visual disability is very common in Stromal Corneal Dystrophies. People with SCDs



are often diagnosed later in life and have a difficulty in accepting the loss of vision. Careful clinical evaluation, genotyping of SCD patients to identify mutations, governmental approval and subsequent development of human clinical trials of possible therapies and treatments should be taken to continue making improvement and effective control of Stromal Corneal Dystrophies.

**GENETIC MONITORING AND EFFECTS OF STOCKING PRACTICES ON:  
*HYPOPHthalmichthys molitrix* POPULATIONS IN PAKISTAN**

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Genetic monitoring in breeding programs is essential for the evaluation of commercializing and disseminating traits in fish species. It provides valuable information for resource managers in the management and conservation of wild and hatchery populations of fish. Genetic degradation, anthropogenic activities and improper stocking practices has raised concern over dwindling in fish biodiversity. Microsatellite markers have been increasingly used in fisheries genetic because of their high applicability in selective breeding programs. This research work was conducted to assess the genetic structure of Chinese carp *i.e* silver carp (*Hypophthalmichthys molitrix*) and also to examine their genetic diversity and population relationship among the population in selected fish hatcheries and natural riverine system in the Punjab Province of Pakistan. At least 50 individuals were genotyped using a total of 7 polymorphic microsatellite markers. Random sampling of juvenile fish was done from the selected sites and transferred to the Aquaculture Biotechnology Laboratory, University of Agriculture, Faisalabad for characterization and genotyping. Different parameters *viz*: allele frequency, heterozygosity, population differentiation, linkage disequilibrium, deviation from Hardy-Weinberg equilibrium and inbreeding coefficient were analyzed by different statistical software include FSTAT, GENEPOP, ARLEQUIN, TFGPA and POPGENE. The value of observed heterozygosity (*Ho*) and expected heterozygosity (*He*) varied between 0.0732-10.428 and 0.415-11.52, respectively. The number of alleles per locus ranged from 4-12. Thirty loci were highly informative ( $PIC > 0.61$ ) in *H. molitrix* population. The deviation from Hardy-Weinberg equilibrium proportions was significant in some locus population. The value of variance among the sampling groups was 3.26% and within the population was 98.02%. UPGMA showed two clusters were formed that exhibited the significant correlation between the geographical distance and the genetic distance. Knowledge of genetic structure of the major riverine and a typical hatchery population is helpful for management of the populations in order to maintain their genetic quality.

**GENETIC HETEROGENEITY OF AUTOSOMAL RECESSIVE MICROCEPHALY IN  
PUNJAB PROVINCE**

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Autosomal recessive Primary microcephaly MCPH (MIM 251200) is a rare neurological disorder characterized by reduced occipitofrontal head circumference (OFC) of at least 4SD with respect to the mean age, sex and ethnic group. Incidence of Microcephaly reported world wide ranging from 1.3 to 150 per 100,000. Occurrence of this disorder is more prevalent in Asian and

Arab societies due to the consanguineous marriages. MCPH is a heterogeneous disorder; so far 16 genetic loci (MCPH1-MCPH16) with mutations in 16 genes have been reported. It is more prevalent in Pakistan, mapping data shows that mutations in 8 causative genes are reported from Pakistani families. The mutation in *ASPM* gene at MCPH5 locus is responsible for more than 50% cases of primary microcephaly in Pakistan. In this study, we ascertained 10 consanguineous families suffering from microcephaly from different regions of Punjab. Blood samples were collected after informed consent. After genomic DNA extraction, genotyping was done with PCR using Oligo STR markers followed by 8% native polyacrylamide gel electrophoresis. One Family MCP-2 showed linkage to MCPH5 locus. Candidate gene *ASPM* has been amplified and sent for sequencing for mutation detection. Two families MCP-1 and MCP-6 are excluded *i.e.* all affected individuals showed heterozygous pattern for all known loci. Genome wide search using SNP array technique and Whole exome sequencing is suggested to search for novel loci/gene or mutations responsible for this phenotype. Results deduced from this study will help in better understanding of molecular basis of this manifestation and its management.

#### **A MOLECULAR GENETICS STUDY ON AN AUTOSOMAL RECESSIVE PRIMARY MICROCEPHALY FAMILY OF PUNJAB**

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The large size of human brain is the most striking characteristic as compared to other mammals. Autosomal Recessive Primary Microcephaly (MCPH) is the most obvious example in which there is one third reduction in brain size as compared to its standard volume with the decline in cognitive abilities. To date 14 MCPH loci has been identified (MCPH1-14) encoding genes *MCPH1*, *WDR62*, *CDK5RAP2*, *CASC5*, *ASPM*, *CENPJ*, *STIL*, *CEP135*, *CEP152*, *ZNF335*, *PHC1*, *CDK6*, *CENPE*, *SASS6* respectively. Pakistani families are highly affected by microcephaly along with other Asian countries because of close kin marriages. *ASPM* affects 50% of Pakistani population. MCPH5 encodings *ASPM* and MCPH2 encoding *WDR62* are mostly prevalent in Pakistan respectively. In the present research, linkage analysis was done in Pakistani family comprising of five generations. Pedigree of MCP1 was constructed by multiple interviews through CYRILLIC and their clinical features were noted. Genomic DNA of 18 members of MCP1 family was extracted through standard methods. Genotyping was carried out by all the known MCPH loci from MCPH1 to MCPH14. Banding pattern was observed through 8% Native PAGE and results showed no linkage with already mapped loci. Genome Wide Search is suggested in order to find the causative gene responsible for autosomal recessive microcephaly in this MCP1 family.

#### **HEIGHT TRENDS IN THE POPULATION OF RABWAH, DISTRICT CHINIOT AND COMPARISON WITH WHO STANDARDS**

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Human Height is a quantitative trait with high heritability ( $h^2=0.6$  to  $0.8$ ), controlled by many gene pairs and influenced by environmental factors such as nutrition, geographical location and

lifestyle. A study was carried out to compare the height trends of the local population with WHO standards. A survey form was distributed to the students of randomly distributed eight schools and two colleges of Rabwah town. The survey form was designed in Urdu explaining the detailed methodology of height measurement and students filled their height information and that of their parents and all siblings above five years. A total 4293 valid observations (male=2084 female=2209) were collected for 5-19 year age group. The smoothing spline technique was applied to analyze the height trends of the collected data. Comparison of male and female height trend indicates that growth rate is almost the same up to 13 years, but it is more for a male after 13 years age. The female height trend revealed no significant increase beyond 16 years, while, for males this trend increased until 19 years. The average height of male adults was 174 cm and for females it was 160 cm. The comparison of percentile curves of local height trends and WHO curves indicates that the local height trends are significantly lower than WHO standard. The 50th percentile curve of the local population was found in the range of the 25th percentile curve of WHO standard. Mid-parental height plots and observed heights of the children show that height trends depend upon parents' height. According to mid-parental height plots 73% male and 84% female data lies within +3 cm range of mid-parental height.

#### **ASH CONTENT AND PROTEIN CONTENT OF MYF-5 GENE ARE ASSOCIATED WITH C6574T MUTATION IN *BOS INDICUS***

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Meat is an important dietary requirement for people and it is consumed globally. Meat industry help to improve the economy of country. Meat quality traits varies in different cattle breeds. The meat quality traits are effected by environmental and genetic factors. The main objective of the proposed study was to find out the novel SNP(s) in MyF-5 Gene and to evaluate their possible effect on meat quality traits in four (04) Pakistani indigenous cattle, *Bos indicus*. Although the study include to determine Allelic, Genotypic frequencies at MyF-5 gene locus and polymorphic information index in Pakistani indigenous cattle breeds (*Bos indicus* cattle breeds). The breeds were Cholistani (CH), Red Sindhi (RS), Dhani (DH) and Thari (TH) Cattle. SNPs were identified and genotyped through PCR-RFLP method and were confirmed through DNA sequencing. In total seventeen (17) SNPs were identified in the bovine MyF-5 Gene. Significant changes in allelic frequencies and genotypic frequencies and medium level polymorphism were detected among four (04) cattle breeds. Least square analysis revealed a highly significant effect of associations between MyF-5 Gene SNPs and meat quality traits. c.C6574T (C>T) Polymorphism has shown highly significant effect for Ash content and Total Meat Protein value ( $P<0.01$ ) but did not shown its significant effect on the pH, Water Holding Capacity (WHC) and total fat content in Pakistani indigenous cattle, *Bos indicus*. Our study ratifies the number of the previously reported substantial effect of correlation of MyF-5 gene with meat quality traits. It is also proposed that this novel SNP c.C6574T could be used as a marker for assortment of animals and can be probably used for cattle breeding using modern methodology, e.g, Marker Assisted Selection (MAS) or Marker Assisted Introduction.

### ATPASE 6/8 GENE ASSISTED GENETIC VARIABILITY STUDIES AMONG THE POPULATIONS OF *LABEO ROHITA*

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A study on the variation amongst the different populations of *Labeo rohita* on the basis of ATPase 6/8 gene was conducted. The present study evaluates the potential of complete ATPase 6/8 region of mitochondrial DNA as a marker region to determine the phylogeography of *Labeo rohita* from fish farm of region Punjab, Pakistan. ATPase6/8 (878 bp) region was used to investigate genetic variation within *Labeo rohita* and develop a global genealogy of genus *Labeo* strains. The ATPase6/8 region was more variable but gave the wide distribution of *Labeo rohita*, the overall levels of sequence divergence were low. Levels of haplotype diversity varied widely among countries with Chinese and India spp. showing the greatest diversity whereas Japanese *Labeo* had undetectable nucleotide variation. Chinese and Japanese carp strains were the most divergent, and their relationships do not support the evolution of independent Asian and European lineages and current taxonomic treatments. The results revealed that 878 bp of ATPase 6/8 region could be a promising marker for determining variations at inter-population as well as intra-population levels in *Labeo rohita*. These results would facilitate conservation and management of this important species.

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### CYP1A1 m1 POLYMORPHISM: GENETIC SUSCEPTIBILITY TO LUNG CANCER IN PAKISTANI POPULATION

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The retrospective study carried out to assess the association of cytochrome P450 CYP1A1 gene with lung cancer. This case-control study employed 100 lung cancer (LC) patients (with male : female of 1:1) and age and sex matched 100 controls (with male : female of 1:1) randomly selected from general population. The MspI polymorphic site also called m1 of CYP1A1 gene is reported to have a strong association with lung cancer in different populations. No study was reported so far from Pakistan describing the association of specific polymorphic site with lung cancer. In order to generate the preliminary data on the polymorphic site and its possible association with Pakistani lung cancer patient the study was designed. The analysis of the CYP1A1 gene polymorphism was performed by RFLP-PCR technique. The data generated was further analyzed using different software to assess potential the risk factor. It was found that the cases have high percentage of mutant alleles (CC+CT) i.e.42% compared to the control 24%. The OR of 3.6 at 95% CI (2.43-5.57) is indicative of fact that the specific polymorphic site is associated with lung cancer patients and is strong risk factor for the lung cancer in studied sample of Pakistani population.

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**ASSESSMENT OF GENETIC VARIATION IN RIVERINE AND HATCHERY  
POPULATION OF GRASS CARP USING MOLECULAR MARKERS**

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Genetic variation is the raw material in species populations which enables them to adapt ecological changes. Fisheries management is facing problem due to over utilization of fish stocks, pollution and various human activities resulting reduction of genetic resources and variations. It is necessary to provide an understanding of the importance of conserving genetic variation both at the level of species and of populations within species. Technologies exist for the assessment of genetic variation at species and population level but utilization of microsatellite markers has been getting importance due to its codominant in nature, Mendelian inherited and high reproducibility. So, this study was conducted to assess the genetic variation of grass carp (*Ctenopharyngodon idella*) utilizing 10 microsatellite markers in Aquaculture Biotechnology Lab. Random sampling was done from selected riverine and hatchery population from different regions of Punjab. The number of effective alleles, polymorphic information content, average and expected heterozygosity were higher in wild population as 4.9, 0.621, 0.789 and 0.891 as compared to hatchery stock. The allelic richness, observed heterozygosity and within-population gene diversity were found to be lower in the introduced populations than in the native populations, presumably due to the small founder population size of the former. Significant genetic differentiation was found between all pairwise populations from different rivers. Consequently, combined effect of all anthropogenic activities, founder effect, introduction history and rapid population expansion help explaining the observed patterns of genetic diversity within and among both native and introduced populations of the grass carp. This analysis would provide frame work for conservation and management of fish stock in riverine as well as hatchery population.

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### 3. HUMAN AND ANIMAL DISEASES

#### PREVALENCE OF REFRACTIVE ERRORS AND ASSOCIATED FACTORS

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The purpose of study is to review and compare the Prevalence of Uncorrected Refractive Errors and associated factors among public school children of Lahore and Debre Markos District, Northwest Ethiopia (AFRICA). Blindness at any stage of life is a concern to public health, however its health burden is significantly aggravated when it occurs at an early stage in life. Childhood blindness limits the child's development, affects quality of life, education and socioeconomic development of the child. According to many studies on refractive error, uncorrected refractive error is one of main cause of visual impairment in children. Failure to treat refractive errors in children may lead to amblyopia which may cause blindness. The World Health Organization has launched the Global Initiative Vision 2020 in 1999 with the slogan "The Right to sight" that has five priority areas. The study regarding the prevalence of uncorrected refractive error and its associated factors among school children in Debre Markos District and public school children of Lahore revealed that Myopia was found the most dominant among all refractive errors. According to Lahore school, the researcher observed that overall prevalence of refractive error was 43 (10.2%). Myopia was found among the most dominant 5.47% followed by astigmatism 1.9% and hyperopic 1.4% in both sexes. Later it was found that 107 out of 540 (19.8%) of the children had refractive errors. Myopia was the most common refractive error being 43% (46/107) of the total. Strong correlation was found between a positive family history of wearing glasses, watching television closely, studying in dim light, over indulgence in computer or video games and low socioeconomic status. It is recommended that adequate preschool examination of the children be made mandatory and part of the admission policy of all the schools in Pakistan and South Africa.

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#### INCIDENCE OF MALARIA, TYPHOID AND CO-INFECTION IN DISTRICT LOWER DIR, KHYBER PAKHTUNKHAWA, PAKISTAN

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Malaria and typhoid fever are among the most endemic diseases in the tropical and developing countries, caused by *Plasmodium* and *Salmonella* species, respectively. Both disease share similar transmission factors and often have similar symptom. The current study deals with prevalence of malaria, typhoid and their co-infection in District Dir Lower, Khyber Pakhtunkhawa, Pakistan from September 2012 to April 2013. This area has favourable environmental condition for the growth of both pathogens. A total of 1889 samples were tested for malaria, typhoid infection and their co-infection by Blood smear microscopy and Typhidot test, respectively. For malarial parasite 117 (38%) samples were found positive, 183 (58%) were positive for typhoid infection, while co-infected cases were only 4% (11). Males were found more susceptible for malaria, typhoid and co-infection as well than females. Similarly it was noted that children of age between 1 to 15 years were more infected than above 15 years. Monthly data revealed that from December 2012 to February 2013, co-infection was absent (0.0%), malarial infection was not observed in January

2013, while typhoid was prevalent throughout the study period. This study provides preliminary information about prevalence of malaria, typhoid and their co-infection but there is a need for further epidemiological study to assess the real risk factors and for developing control strategies of these two medically important diseases.

#### **SURVEY BASED IDENTIFICATION OF GENETIC CONGENITAL EYE MALFORMATIONS AMONG THE POPULATION OF KHAIRPUR DISTRICT, SINDH PAKISTAN**

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Survey based study has been done on eye malformation. Inherited eye diseases were seen in the families of Khairpur District, Sindh Pakistan. Patients were clinically diagnosed from Layton Rehmatullah Benevolent Trust Gambat (LRBT) Khairpur Sindh and re-diagnosed for confirmation from Al-Shifa Trust Hospital Sukkur. Four families having pedigree record of Genetic disorders were selected from the different locations of district Khairpur. Cosanguineous marriages prevalence was seen in all families and disease transmission was found prominently among them generation after generation due to the Homozygosity. There were 10 patients of inherited ocular disease including Anophthalmia, Glaucoma, and Congenital Cataract. When the Patients were identified, their blood samples were collected and send to Khan Research Laboratory Islamabad for genomic analysis.

#### **STUDY OF CONGENITAL ADRENAL HYPERPLASIA DUE TO 21-HYDROXYLASE DEFICIENCY IN PEDIATRIC POPULATION**

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The deficiency of 21-hydroxylase (21OHD) is the most common cause of Congenital Adrenal Hyperplasia (CAH), a disorder of impaired adrenal steroidogenesis. This study focuses on the clinical, biochemical and demographic features of 21OHD CAH patients and aimed to define parameters which will help in management of early diagnosis and severity of genital virilisation in affected females. In this prospective observational study, 66 children with 21OHD CAH presented in The Children's Hospital & the Institute of Child Health, Lahore, Pakistan from 2010-2011 were included. Clinical, biochemical and demographic profiles were obtained and were analyzed using descriptive statistics. Of 66 patients (59 girls and 7 boys), mean age of diagnosis was 2.7 years with

145.46ng/ml as mean value of 17 hydroxyprogesterone levels. Salt wasting, virilizing and non-classical CAH was found in 50.0%, 34.8% and 15.2% patients respectively. History of parental consanguinity was seen in 69.7% of the patients. CAH females were mainly presented by clitoral enlargement (33%) along with labial fusion (29%). In this study of CAH, we observed an extreme elevation of 17OHP. Genotypically female predominance was observed which were previously assigned as male due to clitoral enlargement with labial fusion; however a few cases of ambiguous genitalia were also seen. Whereas very few males were presented with precocious puberty, owing to neonatal death as a result of salt-wasting condition. Classical form of 21OHD CAH is found to be more prevalent. A high rate of parental consanguinity predicts increasing incidence and reveals the need of genetic counseling. Neonatal screening for 21OHD CAH will be a step forward in earlier diagnosis and overcome other social psychological complications particularly in females showing genital virilisation.

#### **BIOCHEMICAL ASSESSMENT OF HYPOTHYROIDISM IN RELATION TO GENDER, AGE FROM HYDERABAD**

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Hypothyroidism is disorder of thyroid hormone which means released too little thyroid hormone, which cause of dry skin, swelled neck, increased sensitivity to cold etc. During the study period 2015, we analyzed with the help of TSH IRMA TUBE and i2000 SR Architect that the 50 patients were suffering from hypothyroidism. Out of 50 patients 15 were male and 35 were female patients 25-35 age group were more suffering and 46-55 were less suffering from hypothyroidism in district Hyderabad.

#### **FOLLOW UP STUDY: THYROID ANOMALIES ARE THE MAJOR CAUSE OF NUMBER OF DISEASES**

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In 32<sup>nd</sup> congress of Zoological Society of Pakistan, I submitted that number of maladies originated under the influence of thyroid glands' physiological derangement. Now ample data is available in its support. I cannot consider the amount of TSH, T3 and T4 concentration in the blood serum, to some extent, it may mislead the case. Attention is given to the auscultatory effects while observing blood pressure, the method devised by Korotkoff, the quality of the rhythm and force of the sound exhibited by blood vessel through stethoscope is considered. Moreover, the pitch of the sound and pulse rate is deliberated. The concentration of missing pulse has not been ignored as well. Muscular jerking or twitching of the muscles of the patients also guides the presence of stress caused by thyroid gland. These situations forced me to speculate that under the influence of thyroid gland THE LUMEN OF THE BLOOD SUPPLYING VESSEL IS REDUCED and the respective organ comes under stress, faces hypoxia and gets weak. (A) In case this stress is on the ARTERIES, the organ goes under nutritive, poorly oxygenated, and this effects the: (i) Nervous system: makes patients psychic, fearful, despondent and may exhibit muscular jerking; (ii)



Heart: Tachycardia, Bradycardia, missing pulse, cardiac arrhythmia with poor sound; (iii) Renal system: recurrent concretions; (iv) Reproductive system: infertility in both sexes and (B) If this vascular stress is on the VEINS, it develops tumours, this type of problem is faced by the young girls in the form breast tumors. A number of patients suffering from above mentioned maladies have been successfully cured through normalizing thyroid gland (by Thyroidenum along with symptomatically related medicines). The remarkable success is, to say, achieving fertility of an Azoospermic person. Under the influence of thyroid gland THE LUMEN OF THE BLOOD SUPPLYING VESSEL IS REDUCED and the respective organ comes under stress, faces hypoxia and gets weak.

#### **SEROPREVALENCE OF BRUCELLOSIS IN SHEEP *OVIS ARIES* IN CHARSADDA, PAKISTAN**

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Brucella spp cause Brucellosis, which is contagious and zoonotic infection of the universe and is a problem of wild and domestic animals particularly ingoats, sheep, buffalos and cows etc. The aim of this survey was to determine the seroprevalence of brucellosis in Sheep in Charsadda Pakistan. 200 samples of sheep *Ovis aries*, Linnaeus (1758), were collected, divided into 3 breed groups (balhe, cross, and local breed) and examined for brucellosis from February to December 2012. Serological examination includes Serum Plate Agglutination Test (SPAT) and Polymerase Chain Reaction (PCR). In sheep 25 (12.5%) were infected by SPAT while 18 (9%) were by using PCR with brucellosis. The prevalence rate of the disease in males and females human samples were determined as follows male, 12% by SPAT while 9% by using PCR, female, 8% by using SPAT while 6% by PCR. The prevalence rate of brucellosis in different age groups, *i.e.*, 0-2, 3-4, 5-6, and >6 of sheep were 14.47, 11.42, 14.28, and 7.69% by using SPAT while 11.84, 7.14, 10.71, 3.84, and 9% by PCR. The prevalence rates of brucellosis in different breeds of sheep, balhe, cross, and local breed were determined as 12.5, 14.81, and 7.69% by using SPAT while 8.75, 11.11, and 7.69% by PCR respectively. The results of the present study indicated that the prevalence of brucellosis in sheep in Charsadda Pakistan is relatively high an effective control program of the disease should be recommended.

#### **THE INCREASING BURDEN OF IRON DEFICIENCY ANEMIA (IDA) IN HYDERABAD, SINDH, PAKISTAN**

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Iron deficiency anaemia is the most prevalent as well as frequent deficiency of nutrition observed globally. The prevalence of this disorder is increasing day by day specially in the developing countries. To determine the prevalence of Iron Deficiency Anaemia at Hyderabad region the cross sectional study was conducted at Diagnostic & Research Laboratory LUMHS Jamshoro/Hyderabad. A total number of 254 patients were collected from January 2015 to October 2015. In which 62% were female patients while 38% were male. The highest prevalence of IDA

was shown in those patients who belongs to the age group of 11-20 years. 61% patients were suffering from severe anaemia, 30% patients had moderate anaemia while only 9% patients had mild anaemia. Reasons may vary, including problems of absorption, nutritional deficiencies or disease. The role of adequate diet and basic nutrition education is very much important to vanquish on this problem.

### SEROPREVALENCE OF CAPRINE TOXOPLASMOSIS IN DISTRICT BAHAWALPUR

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The present study was conducted to determine the seroprevalence of caprine toxoplasmosis during December, 2014-November, 2015. Seventy two blood samples (both from males and females hosts) were collected from local goat's habitating in and around district Bahawalpur. Blood sera were examined for antibodies against toxoplasmosis using latex agglutination test (LAT). Out of 72 hosts 36 hosts were infected with *Toxoplasma gondii*. The overall seroprevalence of *T. gondii* was (45.7%). The parasite was more prevalent (51.8%) in females as compared to (45.7%) males. The prevalence of *T. gondii* was 36% in age group of 1-12 months, 9% in the age group of 12-24 months, 10% in the age group 24-36 months, 5% in the age group in 36-48 months and 7% in the age group of 48-60 months. Blood samples of twenty four (12 infected and 12 non-infected) hosts were tested for hematological parameters. The mean and SEM values of haematological parameters of infected hosts WBC(uL), RBC (uL), HGB (dL), MCV(fL), MCH (pg), MCHC (pg), LYM (uL), LYM, PDW (fL), MPU (fL) P-LCR 10.350±0.837, 2.567±0.283, 13.40±7.87, 23.33±1.56, 96.47±6.61, 31.29±2.85, 32.24±1.62, 56.04±2.81, 5.880±0.64, 8.57±1.95, 14.620±0.289, 68.00±3.76 and mean values of non infected hosts 10.96±1.05, 2.167±0.157, 5.09±1.54, 22.17±1.53, 98.00±5.94, 30.83±4.22, 56.26±2.28, 6.085±0.593, 10.283±0.628, 13.973±0.398, 55.40±5.55 respectively. These means values show a significant difference ((P< 0.05) between infected and non infected hosts. In conclusion the results of the present study showed that the parasite has a significant effect on the haematology of the hosts, it is recommended that further studies on this aspect be done to provide more basic and clinical research information that would improve goats health management.

### HEMATOLOGY, SEROLOGY AND SOCIODEMOGRAPHIC STUDY OF TRANSFUSION DEPENDENT THALASSEMIA PATIENTS IN HYDERABAD AND ADJOINING AREAS

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Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. Patients of transfusion dependent thalassemia (TDT) need frequent blood transfusion, due to which accumulation of iron in blood leads to iron overload. Present study is aimed to study the hematology, serology and socio-demographic characteristics of TDT patients in Hyderabad and adjoining areas. Blood samples of seventy one (71) thalassemia patients were collected. They were

interviewed through a standard questionnaire about their socio-demographic characteristics. The blood was analyzed for hematology and serology by the hematology analyzer SYSMEX KX-21 and chemiluminescence, micro particles immunoassay (CMIA) Architect 1000 SR Abbott. Data was computed by SPSS (version 22) software. Majority of TDT patients were male (104.5%), children of 5-10 years (93.1), having positive family history (106.8%) and uneducated (75%). TDT patients live sedentary life style (95.45%) and consume surface water (61.9%). We explored that the majority (32%) of TDT patients have B+ve blood group. Dietary habits of TDT patients include the white meat consumption (58%), eat 1 to 2 times/week (62%), daily vegetable intake (73%), 5 to 8 cups of tea/week (60.5%) and drink milk/week (66%) and feel less appetite (64.7%). History of blood transfusion of patients reveals that majority of TDT patients take blood 2 times/month (43%) from unknown persons (69%). Majority of TDT patients were hepatitis C positive (64.7%) but very less patients were hepatitis B positive (4.2%) and gone through splenectomy (5.6%). In conclusion, TDT patients are illiterate, consume more tea, and due to blood transfusion from unknown persons they are hepatitis C positive.

#### **ANTICOCCIDIAL SCREENING OF *EUPHORBIA HELIOSCOPIA* LEAVES EXTRACT IN BROILER FARAH**

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Avian coccidiosis is recognized as most detrimental and lethal disease of poultry caused by obligatory intestinal apicomplexan protozoan parasite belonging to genus *Eimeria*. The current traditional methods of controlling coccidiosis mainly depend upon anticoccidial drugs and live vaccine. But their expanded use has resulted in the development of drug resistant strain of coccidian parasite. Because of drug resistance and costly vaccine, interest has been taken in the use of plant extracts for efficient and inexpensive control of coccidiosis. The present study was to figure out the effect of different concentrations of *Euphorbia helioscopia* as anticoccidials in birds compared with coccinil plus anticoccidial drug. A total of 108 broiler (one-day-old) birds were divided into 6 groups (Group 1-6). Each group was in triplicate having 6 birds per cage. The birds were reared for two weeks and fed by coccidiostate free feed. All groups were infected orally with 10,000 oocysts on the 14th day except the non-infected non-medicated control group. From 5-21 days of age three concentrations of 10, 15 and, 20% *E. helioscopia* were orally given once a day. Coccinil plus was given at dose of 0.6g/litre of water. Body weight gain, feed conversion ratio, feed consumption, survival and mortality rate were examined throughout the experimental period. Significant association with P value of ( $<0.001$ ) was noted between weight gain of groups medicated with 20 % *E. helioscopia* and coccinil plus. Oocyst per gram of faeces were recorded on 6<sup>th</sup> to 10<sup>th</sup> day post infection. Lesion score and bloody diarrhoea were recorded during the first week after infection. Serum biochemical parameters such as uric acid, urea, albumin, creatinine ALT, ALP and, AST were investigated in all groups. A significant increase in the level of ALT, AST and decrease in levels of ALP activities was observed. The plasma ALT activity of birds treated at dose of 20% *E. helioscopia* showed significance of ( $P \leq 0.002$ ) with coccinil plus and non-infected non-medicated control groups. Uric acid significantly increases in all herbal medicated groups as compared to the non-infected non-treated group. Albumin and creatinine level decreases in herbal medicated groups as compared to the non-infected non-medicated control group. *Euphorbia helioscopia* leaves extract can be used as anticoccidial agent.

### **SERO-POSITIVITY RATE OF RUBELLA VIRUS INFECTION AMONG PREGNANT WOMEN OF SOUTHERN KHYBER PAKHTUNKHWA, PAKISTAN**

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Rubella is an infectious viral disease causing rash and fever in normal, however in pregnant women cross placenta and directs to infection of a developing fetus causing fetal death, premature birth, abortion or congenital rubella syndrome. A total of 200 Blood samples were collected from pregnant women randomly in different health centers of both urban and rural area of district Lakki Marwat, southern Khyber PakhtunKhwa, in a span of nine months from August 2014 to April 2015. Sera samples were screened for rubella IgG and IgM antibodies by ELISA (enzyme linked immuno-sorbant assay) technique. Our findings revealed that 32(16%) pregnant women were positive for IgG and 5(2.5%) for IgM. Higher sero-positivity rates were found in the age group of 26-33 years. Our result also indicated that women in their second trimester of pregnancy showed highest positivity rate for both IgG and IgM antibodies. In addition to this women residing in rural area showed high sero-positivity rate (IgG=19.7% and IgM=2.91%) than urban area (IgG=7.93% and IgM=1.58%). A lot of women were antibodies positive against RUBV infection suggestive of putting many unborn babies at high risk to rubella infection. key step should be taken by the Government to address this disease in pregnant women.

### **SEROPREVALENCE, RISK FACTORS AND CLINICAL SYMPTOMS ASSOCIATED WITH HUMAN BRUCELLOSIS IN DISTRICT SHEIKHUPURA, PUNJAB PAKISTAN**

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WARDA RIAZ,<sup>1</sup> HIRA HASSAN,<sup>1</sup> TAYYABA KHAN<sup>1</sup> AND SHAHZAD ALI<sup>1</sup>

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The present study was conducted to evaluate the seroprevalence, risk factors and clinical symptoms associated with human brucellosis in district Sheikhupura, Punjab Pakistan. A total of 114 serum samples were gathered from different occupational groups: students, housewives, farmers, labours and others (drivers, painters, and teachers). Data related to gender, age, occupation, locality, urbanicity, socioeconomic status, contact with animals, type of animals, contact with aborted animals consumption of raw milk and brucellosis-related symptoms (fever, sweating fatigue, loss of appetite, chill, arthralgia, headache, anorexia, stiff of neck, back abdomen muscle pain, nausea, body weakness, weight loss) was gathered. The Rose Bengal plate test was performed to evaluate the seroprevalence of brucellosis. The overall seroprevalence discovered was to be 7.9%. Individuals living in rural areas had higher chances of brucellosis seropositivity. This is the first report of human brucellosis related to *B. abortus* in high risk professionals from district Sheikhupura by the use of serological method.

**HUMAN BRUCELLOSIS IN TEHSIL SHARQPUR, PUNJAB, PAKISTAN**

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The present study was conducted to find out the seroprevalence, risk factor and clinical picture associated with human brucellosis in tehsil Sharqpur, Punjab, Pakistan. A total of 200 serum samples were obtained from persons of different occupational groups [Farmer, Housewife, Govt. job, Businessman and others (Students and Labors)]. Data related to gender, age, occupation, locality, urbanicity, socioeconomic status, contact with animal, type of animal, contact with aborted, consumption of raw milk and brucellosis related symptoms (fever, sweating, fatigue, chill, loss of appetite, arthralgia, headache, anorexia, stiff of neck, back/abdominal/muscle pain, nausea, body weakness and weight loss) was obtained on blood sampling date. The Rose Bengal plate test (RBPT) was performed to find out the seroprevalence of brucellosis. The overall seroprevalence was 8%. The individuals living in rural areas and having poor socioeconomic status had higher likelihood of brucellosis seropositivity. This is the first report of human brucellosis from tehsil Sharqpur by the use of serological method.

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**BRUCELLA ABORTUS IDENTIFIED AS THE CAUSATIVE AGENT OF BRUCELLOSIS IN SMALL RUMINANTS IN PAKISTAN**

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The present study was designed to estimate the seroprevalence and identify the causative agent of brucellosis in sheep and goats in Potohar region of Pakistan. Serum samples (n=278) were collected from sheep and goats that had close contact with seropositive cattle and buffaloes herds. Data related to age, sex, location, and breed were collected on the sampling day. Serums were initially screened for presence of Brucella antibodies by Rose Bengal plate test (RBPT) antigens. Seropositive samples were subjected quantitative real-time polymerase chain reaction (PCR) analysis using *Brucella* genus-specific (bcsp31) and *Brucella* species-specific (IS711 for *Brucella abortus* and *Brucella melitensis*) quantitative real-time polymerase chain reactions (qRT-PCR). Twenty-four (8.6%) serum samples were positive by RBPT. Of the 24 seropositive serum samples, 18 (75%) were positive in the *Brucella* genus-specific (bcsp31) and *Brucella abortus*-specific (IS711) qRT-PCR, respectively. *Brucella abortus* was identified as the causative agent of small ruminant brucellosis in Pakistan. Results of this study can be used for the development of an effective control and eradication strategy for brucellosis in livestock, especially small ruminants.

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### SEROPREVALENCE OF BRUCELLOSIS IN GOATS AND SHEEP IN PUNJAB, PAKISTAN

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*Brucellosis* is caused by bacteria of the genus *Brucella* and is characterized by abortion, retained placenta in female and orchitis and infection of the accessory sex glands in males. *Brucella melitensis* is the etiological agent of brucellosis in sheep and goats. The objective of present study was to determine seroprevalence of *Brucella* antibodies in sheep and goats in selected region (Chiniot and Kot Addu) of Punjab Pakistan. A total of 268 serum samples were randomly collected from sheep and goats from Chiniot and Kot Addu. Serum samples were tested for presence of *Brucella* antibodies using Rose Bengal Plate Test (RBPT) according to standard procedure. All total of 16(5.97%) serum samples were found to be positive for *Brucella* antibodies. Seroprevalence was higher in goats (9.90%) as compare to sheep (1.75%). Animals of Kot Addu (11.5%) were found more seropositive than Chiniot. Adults animals were more (8.22%) seropositive than young ones. Male were more seropositive (8%) as compare to female (5.76%). Finally, Local Hairy breed (22.2%) was more seropositive as compare to Daera Din Panah (1.92%) and Beetal (4.54%). In conclusion *Brucellosis* is prevalent in selected areas of Punjab. So, there is dire need of effect control and eradication programme of *Brucellosis* from small ruminants in these areas, to minimize the chance of human *Brucellosis*.

### A HOSPITAL (BINOR) BASED RETROSPECTIVE STUDY OF THYROID CANCER IN SOUTHERN DISTRICTS OF KHYBER PUKHTOONKHW PAKISTAN

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The incidence of thyroid cancer is increasing in developing as well as developed countries each year. Therefore this study was executed to estimate the incidence of thyroid cancer from southern Districts of Khyber Pakhtoonkhwa (KP). The data were collected from Bannu Institute of Nuclear Medicine, Oncology and Radiotherapy (BINOR) KP. The overall thyroid cancer found in males and females was 49(37.12%) and 83(62.87%) respectively. The gender wise analysis indicated that in males the highest incidence 6(46.1%) was recorded in 2009 whereas lowest 2(20%) in 2015. In females, the highest incidence 8(80%) was recorded in 2015 whereas the lowest 7(53.8%) in 2009. The district wise analysis showed that maximum patients 61(46.21%) hailed from district Bannu whereas minimum patients 1(0.75%) hailed from district Tank. Age wise analysis revealed that 21-40 years age group was most prone to thyroid cancer with 63(47.7%) cases whereas the age group 81-100 years was the less affected with 1(0.75%). The anaplastic cancer was found higher 3(75%) in males compared to females 1(25%), whereas Papillary and

Follicular carcinoma were found higher 15(78.9%) in females compared to males 4(21%). The increasing trend in cancer ratio was observed among females whereas gradual decline was noticed among males from 2009 to 2015. The severe scenario can be controlled by increasing awareness among the community which will lead to instant and immediate diagnosis to avoid chronic cancer. Government efforts are needed to assist the community for low cost treatment.

#### **EPIDEMIOLOGY OF ASTROVIRUS AND SEROTYPES DIVERSITY IN DISTRICT KOHAT, KHYBER PAKHTUNKHWA**

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In the current study a total of 10/220 (4.54%) patients were tested positive for Astrovirus (AstV). Children less than 5 years have the highest risk of AstV. AstV was more frequent in male as compared to female. AstV infected individuals which show that the serotype 1 is dominant as compared to other serotypes. The study reveals the population based scrutiny data to estimate illness occurrence. Estimating incidence and outcomes of AstV disease is critical to establish the burden of AstV in district Kohat, KPK, Pakistan.

#### **EPIDEMIOLOGY RISK FACTORS FOR ORAL CANCER DISEASE: A CASE CONTROL STUDY**

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Studies have shown that an annual oral examination carried out by a primary care dentist can detect mucosal abnormalities that are unknown to the patient. Which is attributed to the local custom of chewing tobacco products such as paan, gutka and naswar. These things are widely used in Pakistan mainly by the lower socioeconomic group. They pose a high risk for oral cancer. The continuous exposure to corrosive condiments causes abrasions of oral mucosal surface making it more susceptible for virulent strains to gain entry into the basal cells. Present study was aimed to explore the epidemiological risk factors for oral cancer disease in cancer patients treated at NIMRA Hospital, Jamshoro. The study design was a Case study and was conducted at NIMRA Hospital, Jamshoro. 67 male and 30 female patients of ≤15 to above 75 years age group were admitted treatment of oral cancer. Oral cancer patients was asked for the help of a questionnaire designed to know the age, sex and month of presentation of oral cancer diseases. Total no of 97 Oral Cancer (OC) patients admitted at Liaquat University Hospital, Hyderabad and control group was composed of 110 (age and gender matched with negative personal and family history of any cancer), all were investigated by giving them a standard questionnaire specially designed to study the risk factors for oral cancer disease. The majority of OC Patient were male and married, labour, having 31-45 year age, sindhi speaking and were underweight. We found alcohol, smoking, paan, mainpuri, Supari,

gutka, naswar and collective addiction were significantly positively associated with OC disease. Less consumption of meat, more tea intake, and deep fried food consumption using khula pakwan and ghee are significant risk factors for OC disease. Vegetables, fruits, fried food, sweet food and saltish food were not associated with oral cancer disease. In conclusion, males, 31 – 45 years age group, mainpuri, paan, smoking, gutka, khula pakwan oil, less consumption of meat, more intake of tea and deep fried foods may be the risk factors for OC disease.

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#### **EFFECT OF RADIOTHERAPY ON HEMATOLOGY OF BREAST CANCER PATIENTS TREATED AT NIMRA, JAMSHORO**

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Breast cancer is the malignancy of the breast tissue which is the most frequently diagnosed cancer in the women worldwide. In Pakistan, the most frequently diagnosed cancer among females is also breast cancer, accounting for nearly one in nine female patients. The epidemiological data has shown several risk factors associated with breast cancer. Radiotherapy may has effects on hematology of breast cancer patients. Hence, the present study was conducted to see the effect of radiotherapy on hematology of breast cancer patients treated at NIMRA, Jamshoro. The blood samples of 30 pre treated (prior to radiotherapy) and 30 post treated(after completing radiotherapy in 6 cycles at NIMRA, Jamshoro) breast cancer patients were collected from NIMRA and used for the analysis of Urea, Creatinine, Complete blood count (White blood cell count (WBC or leukocyte count), WBC differential count, Red blood cell count (RBC or erythrocyte count), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin(MCH), Mean corpuscular hemoglobin concentration (MCHC), and Platelet count. Results revealed that the levels of WBCs, RBCs, Platelets, and Monocytes count were significantly decreased in post treated Breast Cancer patients as compared to pre treated Breast Cancer patients. We found no variation of MCV, MCH, MCHC, Neutrophils, Lymphocytes, Eosinophils, and Basophils in pre treated Breast Cancer patients as compared to post treated Breast Cancer patients. We found increased urea and decreased creatinine in pre treated Breast Cancer patients as compared to post treated Breast Cancer patients. Age wise comparison of pre treated Breast Cancer patients showed no significant variation among all age groups except serum urea. Whereas, the age wise comparison of post treated Breast Cancer patients revealed significant difference among all age groups for MCV, MCH, MCHC, and RBCs count. In conclusion, Radiotherapy has adverse effects on hematology of breast cancer disease.

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#### **SERUM LIPID ALTERATIONS IN PATIENTS TREATED WITH SECOND GENERATION ANTIPSYCHOTIC DRUGS AT SIR COWASJI PSYCHIATRY HOSPITAL, HYDERABAD**

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The second-generation antipsychotic drugs (SGAs; also known as “atypical antipsychotics”) has enabled effective treatment of psychotic disorders while reducing the risk of extrapyramidal



side effects. However, they have been associated with greater incidence of metabolic changes such as hypercholesterolemia, hypertriglyceridemia, increased incidence of type 2 diabetes, hypertension and increased general myocardial infarction risk ETC. To analyze the lipid alterations in patients treated with SAGs we undertake the present study. We randomly selected 40 Psychotic inpatients using antipsychotic drugs (Clozapine, Olanzapine, and Risperidone) from CJ Psychiatry Hospital, Hyderabad. 5 ml fasting blood samples were collected on admission and after each month (for 3 months) during treatment, along with age and gender matched 47 controls having negative personal or family history of psychotic disorder, obesity, diabetes and cardiovascular diseases. Collected blood samples were analyzed for serum lipid alterations. Significantly decreased concentration of Total Cholesterol, HDL-C and Total Lipids were found in the psychotic patients as compared to controls. Significant increased concentration of all the lipid contents except HDL-C (significantly decreased) were found when patients were compared before baseline and after treatment of 3 months. In conclusion among three antipsychotic drugs it was found that Clozapine is majorly responsible for causing the Lipid alterations.

#### **TO DETERMINE THE EFFECT OF TEMPERATURE ON *ANOPHELES* MOSQUITO POPULATION AND MALARIA TRANSMISSION IN SINDH PAKISTAN**

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*Anopheles* mosquitoes are the vectors of malaria; they transmitted malarial parasites from infected individuals to a healthy person. The mosquito population ratio is a key determinant of malaria risk. Life cycle and survival rates of *Anopheles* mosquitoes and *Plasmodium* parasites that cause malaria depend on temperature. During present study period (2014), it was observed that the population of *Anopheles* mosquitoes had two peaks at first they started to increase from the mid of March to mid of May and its second activity started from the end of July and reached to its peak in September due to favorable temperature then gradually decline from November because of low temperature.

#### **AN OUTBREAK OF CUTANEOUSE LESHMANIASIS IN LOCAL POPULATION OF DIR UPPER, PAKISTAN**

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Leishmaniasis is an infectious disease caused intracellular parasitic protozoans belonging to a genus of flagellate protozoa called *Leishmania*. More than 25 species of genus *Leishmania* are present worldwide in which several species are pathogenic to human. In many cases cutaneous infection is formed but some species cause subcutaneous or deeper tissues and visceral infection. Cutaneous leishmaniasis is a skin disease caused by *Leishmania tropica*. This disease is transmitting

by sand fly. The disease is endemic along entire Western border of Pakistan. During the present research the samples were collected from infected peoples of different area of Dir Upper. Total 548 samples were collected among which 346 (63%) were positive cases. In infected peoples 297 (86%) were from local population and 59 (14%) from Afghan refugees. Total 235 (68%) males and 111(32%) females were effected. The high prevalence rate (48.5%) was noticed in the age of 11-20 years old peoples. The prevalence rate between the age of 0-10 was 29.1%. The low prevalence rate (13.6%) was notice in the peoples over 30 year. Mostly one lesion was noticed in effected people (69%). The patients with two lesions were 22%. The numbers of effected persons with more than two active lesions were little in number *i.e.* 8.3%. Most of the lesions were reported on face (36.1%). The second effected part of body noticed in the study was hand (26.5%). Most of the lesions were dry (81%). It is concluded that high prevalence rate was observed. People's awareness is very necessary.

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### **ROLE OF CASSIA FISTULA LEAVES EXTRACT FOR THE CONTROL OF COCCIDIOSIS IN BROILER CHICKS**

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Avian coccidiosis one of the most common and economically devastating intestinal disease caused by the protozoan parasite of genus *Eimeria*. For over 60 years anticoccidial drugs have been used to combat infection but their extensive use has resulted in the development of drug resistant *Eimeria Spp.* strains. Plants products having antioxidant properties can be used for the control of coccidiosis due to the association of coccidial infection with lipid peroxidation of the intestinal mucosa. In the present study, the comparative efficacy of salinomycin and 70% ethanolic extract of *C. fistula* leaves as a botanical coccidiostat were investigated. Total 108, day-old chicks were randomly divided into six groups (n=6), each group having triplicate. At 14 day of age, eight groups were infected with oocysts of *Eimeria* spp. at 10,000 per chick. From 5<sup>th</sup> day post inoculation groups A-F were orally supplemented with 10, 15, 20% *C. fistula* leaves extract. Group H was kept as negative control, treated with 0.6gm/litre coccinil plus. Treatment was given for seven days. Throughout the experimental period the body weight gain, feed consumption, feed conversion ratio were investigated while bloody diarrhoea and oocysts excretions were investigated at the first and the second week after infection. McMaster counting technique was used for determining the number of oocysts egg per gram of faeces. Groups treated with 0.6gm/litre coccinil plus and 20% *C. fistula* had significantly higher ( $P \leq 0.001$ ) body weight gain compared to infected non- medicated control group. 20% *C. fistula* resulted in feed conversion ratio similar to coccinil plus medicated group. Excreted oocysts per gram of faeces were lower in the groups treated with 15%, 20% *C. fistula* than in the infected control group. Bloody diarrhoea of mild extent comparable with a standard coccidiostat *i.e.*, coccinil plus, was shown by group receiving 20% *C. fistula* as compared to infected non-medicated group. In addition, *C. fistula* leaf extract at 20% had survival rate of 100 % after infection with *Eimeria* spp. Blood samples were collected from each group. A significant decrease in albumin, urea, creatinine and alkaline phosphatase (ALP) activity was observed while increase in ALT, AST was evident in coccidian infected chickens as compared with infected non-medicated control group. Current investigations revealed the anti-coccidial potentials of *C. fistula* leaves extract. Synthetic coccidiostats can be replaced by phytochemicals thus reducing risky effects of previous ones.

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### SEROPREVALENCE OF *TOXOPLASMA GONDII* IN PET DOGS OF PESHAWAR, PAKISTAN

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*Toxoplasmosis* is a multicultural zoonotic infection caused by specie called *T. gondii* that infect large range of hosts like cattle, pets and human. The transmission's way in herbivorous animal is through ingestion of sporulated oocysts. Though the infection can also be transmitted to omnivorous and carnivorous through ingestion of tissue cysts present in undercooked meat. The cats are the natural reservoir in which the oocysts are fashioned during sexual phase of life cycle. Dogs get infected with oocysts voided from cats, definitive hosts of *T. gondii*. In present study sera samples of 100 dogs from district Peshawar were tested for anti-*T.gondii* antibodies: IgG and IgM through enzyme-linked immunosorbant assay (ELISA). Twenty dogs (20%) were found positive for both IgG and IgM. Seropositivity was (11.11%) in male and (42.85%) in female dogs. Age-wise older dogs were more positive than younger dogs for *T.gondii*. Breed wise the prevalence rate was higher in Libra dog () than the other breeds of the study. Our findings suggest that routine screening tests of pets' dogs should be practiced by the dogs owners and they should be informed about the zoonotic aspect of this disease.

### PREVALENCE OF MALARIAL DISEASE IN LOCAL POPULATION OF TEHSIL BARIKOT, SWAT

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During the present study, prevalence of malarial disease were carried out in local population of Tehsil Barikot, District Swat. Blood samples were collected during May to November, 2014. A total of 997 individuals were screened for malarial test in tehsil Barikot including urban and rural area. A performa was designed including the past history of the individual *i.e* age, sex, education, domestic animal and socio-economic conditions. Samples were divided into four category of age *i.e*. (1-15), (16-30), (31-45), (46-onward), gender wise, union council (Kota, Barikot, Shamoza and Ghalegi) and month wise. Thick and thin blood smear were prepared and examined under microscope. In a total of 997 samples, 204 (20.46%) were found positive, in which mostly under the age of 15 year (23.36%) and less cases were recorded in adult (46 and onward) which was 22.64%. Most of the positive cases were found in the month of November which are 24 out of 67 (35.82%) and less cases were noted in the month of May which are 12 out of 106 (11.32%). Most cases were found in U.C Kota 230 blood sample were collected out of 60 were positive (26.08%) and low prevalence in U.C Ghalaigai, a total of 320 sample, 42 were positive (13.12%). The

Gender wise prevalence of malaria is more found in male than female. Male was 106 out of 385 (27.53%) and female 98 out of 612 (16.01%). It is concluded that *P. vivax* is the most common in tehsil Barikot.

#### **EFFECT OF *PLASMODIUM VIVAX* DENSITY ON WBCS AND PLATELETS COUNTS IN MALARIA PATIENTS OF DISTRICT BANNU KHYBER PAKHTUNKHWA PAKISTAN**

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This study was conducted in District Headquarters Teaching Hospital and Women & Children Teaching Hospital District Bannu to correlate the *P. vivax* density with WBCs and platelets count in malaria patients. A total of 137 suspected individuals who visited hospitals with the complaints of fever, headache, abdominal pain and shivering. Among these 49 were found positive for *P. vivax* and 88 were found negative. The patients, who have less than ten years of age, were more infected by *P. vivax*. The maximum density in patients was recorded four per field in microscope but the density two were more abundant. The parasite density decreased with the increase in WBCs count. Similarly the platelets count increases when parasite density decreases.

#### **IN SITU, LATEX AGGLUTINATION FOR SEROPREVALNCE OF TOXOPLASMOSIS IN PASTORAL HERDS OF GOATS AND ATTENDENTS IN PREMISIS OF FORT MUNRO PUNJAB, PAKISTAN**

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Toxoplasmosis is a protozoan disease of cats; however, all warm-blooded animals comprising mammals, birds and human beings can be infected with Toxoplasmosis because of its zoonotic and foodborne nature. Although it has a widest range of intermediate hosts yet there is only one species; *gondii* under the genus *Toxoplasma*. Infection in one-third human population indicates that since the time of its discovery, *Toxoplasma gondii* is remarkably a successful protozoan that is endemic and distributed worldwide. The intention of this study was to observe the prevalence, potential risk factors of Toxoplasmosis in pastoral goats and herd attendants in Fort Munro (D.G.Khan) Punjab, Pakistan. A cross sectional study was conducted on 52 pastoral herds of goats and herd-attendants during March 2013 to July 2013. Aggregate of 237 goats from 52 pastoral herds and their 92 male herd-attendants were screened using Latex agglutination test.

Overall 27% prevalence was recorded in goats, 69% in pastoral herds and 19.6% in herd-attendants. The prevalence of disease was (14.77%) in Pahari and (12.24%) in Hairy goat breed whereas (21.52%) in female and (5.49%) in males. Sero-positivity trend was correlated with increase in the age of goats and attendants. Natural water reservoirs, presence of cats and dogs in herds, offering raw meat offal to cats and dogs and pastoral grazing itself were significant risk factors of toxoplasmosis for goats. Risk factors like, medium-cooked meat, eating unwashed fruits and raw vegetables and access of cats to water and food were significantly associated with toxoplasmosis in herd attendants. It is concluded that disease burden of Toxoplasmosis is increasing gradually and globally. It can be reduce in human beings and animals by reducing risk factors and maintaining proper hygienic measures.

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#### **EPIDEMIOLOGY OF HEPATITIS B VIRUS (HBV) INFECTION IN IDPS OF WAR AGAINST TERRORISM IN NORTH WAZIRISTAN AGENCY PAKISTAN**

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Hepatitis B virus is the major cause of liver infection that includes hepatocellular carcinoma and cirrhosis. Nowadays 350 million people suffer from chronic infection and 2 billion people are infected from Hepatitis B virus. However in Pakistan HBsAg carrier range from 4%-10% while 23% are immune to HBV, 34% show exposure and 3.5% children are positive carriers. The objective of the study was to determine the epidemiology of hepatitis B virus in IDPs of war against terrorism in North Waziristan agency Pakistan that are settled in district Bannu. A total 1837 blood samples were collected from IDPs of District Bannu region including IDPs of various ages in which 1115 (60.69%) were males and 722 (39.30%) were females and their ages were ranged from 1 year to above 60 year. These blood samples were taken from different regions of District Bannu where IDPs were settled and were screened for HBsAg using immunochromatography (ICT) method and enzyme linked immunosorbent assay (ELISA) method. Their blood samples were screened with ICT kits 237 (12.90%) IDPs samples were infected and positive while remaining 1600 (87.09%) were healthy and negative for HBsAg. When these all 1837 blood samples were re-examined with ELISA then 249 (13.55%) IDPs samples were infected and positive for HBsAg while 1588 (86.44%) were negative for HBsAg. ELISA is more sensitive and diagnosing method than ICT (Immunochromatography test) kit method.

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#### **PREVALENCE OF MALARIA IN IDPs OF WAR AGAINST TERRORISM IN NORTH WAZIRISTAN AGENCY**

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Malaria is still the most prevalent infectious diseases and is responsible for enormous human morbidity and mortality. The disease one of the biggest killer of the world, especially in pregnant women and children. It account for approximately one million deaths each year. Two species of *Plasmodium* namely the *Plasmodium vivax* and *Plasmodium falciparum* are reported in Pakistan.

This study was conducted with the main aim to identify the prevalence of malarial infection in TDPs/IDPs of human population settled in district Bannu. Blood samples were collected and prepared thick and thin blood film on same slide and detected only by microscopic technique. Out of 374 suspected cases of malaria, 32.62% were found to be positive for malarial parasite in blood smear slides. Out of positive cases, 87.70% were identified as *Plasmodium vivax* infection, 12.29% cases with *P. falciparum*. *P. vivax* is predominant then all species of *Plasmodium* and more prevalence in males then female.

#### **FREQUENCY OF HEPATITIS C VIRUS INFECTION IN DIABETIC PATIENTS OF KHYBER PAKHTUNKHWA, PAKISTAN**

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Hepatitis C is a worldwide public health problem causing chronic liver diseases and affects 180 million people globally. We determined the frequency of hepatitis C virus infection in diabetic patients in various divisions of Khyber Pakhtunkhwa province, Pakistan. We analyzed the subject's data for blood sugar and screened for anti HCV by ELISA and HCV RNA by PCR. For statistical analysis SPSS version 14.0 for windows was used. Chi-square test was used to explore proportional relationship. The level of statistical significance was set at  $P < 0.05$ . A total of 700 diabetic patients of age ranges from 30-70 years, were studied. Of the total, 11.71% were found positive for HCV antibodies, of which 7.43% were confirmed for HCV RNA. High rate of infection (13%) was observed in Peshawar and lowest (5%) each in Hazara and Malakand. Similarly, higher rate of infection of HCV in diabetic patients was observed in the age group of 46-60 years. Our finding shows that Hepatitis C virus circulates in the diabetic patients in various regions of Khyber Pakhtunkhwa province. HCV infection have statistically significant relationship with old age diabetic patients ( $\chi^2 = 22.58$ ;  $df = 2$ ;  $P = < 0.05$ ).

#### **PREVALENCE OF PLASMODIUM INFECTION AMONG THE HOSPITALIZED PREGNANT WOMEN IN DISTRICT BANNU KHYBER PAKHTUNKHWA, (KP) PAKISTAN**

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The present study was conducted in Women & Children Teaching Hospital (WCTH) District Bannu to analyze the plasmodium infection among the pregnant women. For this study those pregnant women were selected who visited the hospital with the complaints of high fever, shivering, vomiting and headache. A total of 250 blood samples were collected, among these 57 (22.80%) blood samples were found positive and 193 (77.20%) were negative. Of the positive blood samples 52 (20.80%) were infected with *P. vivax*, while 5 (2.00%) blood samples were found to be infected with *P. falciparum*. Women of the age group 20-30 years were more susceptible to

the plasmodium infection. In most of the patients plasmodium density one was recorded. Women of the rural area were more infected than the urban area.

#### **PREVALENCE OF ACTIVE HEPATITIS C VIRUS INFECTION IN GENERAL POPULATION OF KHYBER PAKHTUNKHWA, PAKISTAN**

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Hepatitis C virus (HCV) is a major public health problem and is the leading cause of chronic liver disease. It has affected an estimated 180 million people globally. In Pakistan an alarming rate of HCV outbreaks have been reported but a very limited data regarding PCR based prevalence studies is available. PCR has emerged as the most powerful diagnostic tool for the detection, quantification and genotyping of HCV RNA in the blood. In view of the above facts, the present PCR based prevalence study was designed with main aim to detect active HCV infection and to observe the possible risk factors responsible for HCV infection in various divisions of the Khyber Pakhtunkhwa province, Pakistan. A total of 2534 individuals (1748 males and 786 females) were screened for anti HCV antibodies by ELISA (EIA) method. Among 2534 individuals 295 (11.64%) were found positive for anti HCV antibodies. All the anti HCV antibodies positive samples were analyzed for HCV RNA by nested PCR. Chi-square test was used to explore proportional relationship. The level of statistical significance was set at  $P < 0.05$ . Of the total 2534 samples, 202 samples (7.97%) were found positive for HCV RNA. The 202 HCV RNA positive individuals included 132 males and 70 females. Higher prevalence of active HCV was reported from Kohat division (9.40%), followed by Hazara division (8.63%), D.I.Khan division (8.40%), Mardan division (8.35%), Peshawar division (7.45%) and Bannu division (7.44%). The lowest prevalence of active HCV was reported from Malakand division (6.32%). Most of the people with age group 16 to 30 years were infected as compared to other age groups. Risk factors observed in HCV positive individuals were unhygienic barber practice, blood transfusion, general and dental surgery, unsafe injection and sharing personal items. Trend of sharing personal items was common. There was found statistically significant relationship between HCV infection and the risk factors ( $\chi^2 = 25.98$ ;  $df = 8$ ;  $P = 0.0011$ ). Further studies needed to investigate the various genotypes of HCV in the study area.

#### **INCIDENCE OF INFECTIOUS BRONCHITIS DISEASE IN COMMERCIAL BROILER BIRDS OF HYDERABAD DISTRICT, SINDH, PAKISTAN**

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The present study was conduct to study the incidence of infectious bronchitis disease in commercial broiler birds in five talukas *i.e* Hydearbad, Hala, matyari, T. M Khan and Tando

Allahyar, of Hyderabad district. Total number 979 of birds was found affected in all talukas out of which 756 (77.22%) was died and 223 (22.77% ) was survived from IBV in all five talukas of Hyderabad district . The data was collected randomly by personal survey of five talukas of Hyderabad District. The samples were brought at poultry disease diagnosed laboratory Hyderabad for confirmation of diseases. The disease was diagnosis on the sign and symptoms and postmortem was conduct for the confirmation of the disease. The present result shows that the IBV was remarkably higher no. of affected ( $21.66 \pm 1.36$ ) in Hyderabad among all talukas while highest mortality rate was observed in Hyderabad ( $16.75 \pm 1.30$  with 22.69%) and lowest at talukas hala ( $10.66 \pm 0.94$  with 23.355%) and high no. of birds survived in taluka Hyderabad ( $4.91 \pm 0.55$  with 22.69%) among in all talukas of Hyderabad district. It may be concluded from this study that the prevalence of disease is possibly be control by hygienic measurement, bio security and also Vaccination is the principal method of control.

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**EPIDEMIOLOGY OF HEPATITIS C VIRUS INFECTION IN IDPs OF WAR AGAINST  
TERRORISM IN NORTH WAZIRISTAN AGENCY, PAKISTAN**

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HCV infection appears to be endemic in most part of the world, with a prevalence of around 3% world-wide. HCV infection is life astounding health problems worldwide, with over 170–200 million infected people including about 17 million from Pakistan and it causes cirrhoses and other complications that often lead to death. More than 350,000 people die from hepatitis C related liver diseases every year. The study was conducted with the main aim of determining the epidemiology of hepatitis c virus infection in IDPs (internally displaced people) of war against terrorism in North Waziristan Agency, Pakistan. They were screened for HCV by rapid method (ICT). The study was conducted from September, 2014 to August, 2015. A total of 1837 individuals were sampled including males and females of 1 – 70 years of age. Out of these 516 individuals (28.08%) including 337 (30.22%) male and 179 (24.79%) female were found positive for HCV. They were categorized into 5 age groups. The prevalence rate of HCV infection is directly related with age *i.e.* higher the age group, higher will be the rate of infection. In this study the age group of >60 has high rate of infection. Individuals with the risk factor of dental surgery also have the high prevalence. The HCV infection is relatively higher due to lack of education and awareness about the risk factors of the disease, shortage of medically qualified and scientifically trained health care workers and lack of health instruments.

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#### 4. MICROBIOLOGY

##### EFFECT OF ASCORBIC ACID ON SURVIVAL AND BACTERIAL CONTENTS IN THE GUT CONTENTS OF *OREOCHROMIS NILOTICUS*

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Studies were conducted to evaluate the dietary impact of Ascorbic acid on the survival and bacterial load in the gut contents of *Oreochromis niloticus*. Fish stocked in the earthen ponds which were to be marketed for human consumption was taken and the Vit C as a source of ascorbic acid was added in the standard feed for evaluation. There were three tanks in replicates selected as control, treatment 1 (T<sub>1</sub>) with 3% addition and treatment 2 (T<sub>2</sub>) with 5% addition of ascorbic acid, respectively. After a ninety day trial results 100% survival was observed in all the replicates were analyzed by statistical software SAS and analysis of variance showed significant differences among the treatments. The highest microbial load was observed in control (1.12E±07 to 1.67E±06) while in the treated tanks it was observed as 1.03E±05 to 1.23E±06 in T<sub>1</sub> and 7.10E±05 to 9.8E±05 in T<sub>2</sub>.

##### OPTIMIZATION OF MEDIA FOR PROTEASE PRODUCING *BACILLUS SUBTILIS* USING RESPONSE SURFACE METHODOLOGY

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Protease enzymes hold prominent position because of their wider range applications in various industrial fields. Among bacteria, the *Bacillus* species are known as excellent source for the enzyme production due to simpler nutritional and metabolic requirements. In this study, *Bacillus subtilis* MH1 strain was used for the production of extra cellular alkaline protease. The optimization of medium parameters for optimal cell growth and protease production was achieved through central composite design (CCD) using response surface methodology (RSM) technique. Four input variables viz. casein, glycerol, yeast extract and MgSO<sub>4</sub> were selected for optimization. The software generated 30 experimental runs on the basis of random selection of variables. The effect of different levels of variables was evaluated statistically through application of response surface methodology and design experiment software. The maximum enzyme yield and cell growth was observed to be 462 mg/L and 0.820 g/100 ml respectively. The optimum level for bacterial growth and protease production of casein was found to be 1.15 g/250 ml while that of glycerol was noted to be 4 ml/250 ml. Similarly, the yeast extract level 1.25 g/250 ml resulted in maximum enzyme yield and bacterial growth. The best concentration of MgSO<sub>4</sub> was detected to be 0.125 g/250 ml. The p-values generated through statistical analysis by ANOVA revealed that glycerol and yeast extract were most significant variables for the enzyme production. The evaluation of interactive effect of variables showed that combination of casein-casein, glycerol-glycerol, yeast extract-yeast extract, MgSO<sub>4</sub>-MgSO<sub>4</sub>, casein-glycerol, glycerol-yeast extract and glycerol-MgSO<sub>4</sub>

interactive effect showed statistically significant values. Likewise, the interaction between casein-yeast extract and yeast extract-MgSO<sub>4</sub> was rendered significant for the optimal bacterial growth.

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### ISOLATION OF MEDICALLY IMPORTANT FUNGI FROM BODY SURFACES OF COCKROACHES IN LAHORE

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Cockroaches are found as medically important pests of human habitation that are causing a serious public health problem. They may harbor fungal spores on their external body surfaces, crevices and cracks between thorax and head and disseminate on human food and cause serious diseases, food poisoning and allergies. Hence, they are regarded as microbial vector in human habitation. This studies focus on role of cockroaches in transportation and dissemination of pathogenic fungal spores to human food. Totally, 200 adult cockroaches were collected from houses, food stores and offices directly by hand and by sticky traps and food-baited traps. The cockroaches were collected into sterile universal containers and transported to the laboratory. Suspensions were made by shaking cockroaches in normal saline and cultured on Sabouraud dextrose agar and Potato dextrose agar plates. The organisms were identified by First Fungal Culture Bank of Pakistan on the basis of microscopic view of conidia and hyphae. Common fungal contaminants found on external surfaces of cockroaches include *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus zonatus*, *Geotrichum candidum*, *Mucor spp.*, *Rhizopus spp.* and *Metarhizium spp.* *Aspergillus flavus*, *Rhizopus spp.* and *Geotrichum candidum* were most prevalent among all cockroaches. All isolates shows significant growth in laboratory. These saprotrophic, pathogenic isolates produce aflatoxins and aspergillosis and geotrichosis in immunocompromised individuals. Cockroaches are uniformly distributed in human habitation which can be a possible vector of pathogenic fungal spores which can cause food poisoning and illness. Some entomopathogenic isolates can be used as fungal biocontrol agent of insect pests of agriculture crops.

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### SCREENING OF INDIGENOUS ACTINOMYCETES, ISOLATED FROM HIMALAYAN MOUNTAIN RANGE PAKISTAN, AGAINST VARIOUS MDR BACTERIAL PATHOGENS AND MYCOBACTERIUM TUBERCULOSIS

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Characterization and screening of the indigenous actinomycetes isolates, isolated from Himalayan Mountain Range Pakistan for novel bioactive compounds against various MDR bacterial pathogens and *Mycobacterium tuberculosis*. 144 actinomycetes isolates, isolated from different soil samples of Himalayan Mountain Range Pakistan, were initially screened biologically by agar diffusion method against non-pathogenic gram positive and gram negative bacteria for determining their antimicrobial potential. Then the selected isolates were screened by both agar diffusion and well diffusion method against the different MDR bacterial pathogens i.e *P.*

*aeruginosa*, *Proteus mirabilis*, *K. pneumoniae*, *S. aureus*, *E.coli*. The antimicrobial activity of these isolates was also checked against *Mycobacterium tuberculosis* on LJ medium by proportional method. Cytotoxicity assay of the extracts of these isolates was performed against *Artemia salina* by brine shrimp micro well cytotoxicity assay. Chemical screening of the crude extracts was done by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The selected isolates were characterized by different biochemical and physiological tests. Antimicrobial activity of the 42 isolates was very promising against MDR bacterial pathogens. Some of these isolates are CTF 18, CTF 14, MMG 35, MMG 36, KS 32, KS 30, KS 2, KS 84, KS 14, KS 27, KS 42, 27 b, KS 59 etc. The results were also very encouraging for antimycobacterial activity. TLC chromatograms under UV and staining with different staining reagents exhibited different colored bands which indicated diversity of the secondary metabolites produced by these isolates. The HPLC chromatograms of these isolates indicated various peaks at different retention time, supported the results of the TLC. Antimicrobial activity of the selected actinomycetes isolates is very impressive against MDR bacterial pathogens and *Mycobacterium tuberculosis*. Further studies include isolation, purification and structure elucidation of specific bioactive compounds from the crude extracts of these isolates by different chromatographic analysis, mass spectrometry and NMR. This may lead to the development of new antibacterial substances.

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#### BIOACTIVE ACTINOMYCETES FROM CHENAB RIVER SEDIMENTS: SCREENING FOR ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS

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These days mortality, morbidity and health cost have been increased due to multiple antimicrobial drug resistance in pathogens across the world. So it is important to discover new active compounds. This study was designed to screen the Chenab River actinomycetes for antimicrobial activity and cytotoxicity. The water and mud samples of Chenab River were collected from the Sialkot region (Punjab, Pakistan) and were processed for the selective isolation of actinomycetes. The isolated strains were identified by morphological, biochemical, physiological characterization and by 16S rRNA gene sequencing. The selected strains were screened for antimicrobial activity and cytotoxicity using agar plug method and agar diffusion assays and by microwell cytotoxicity assay against *Artemia salina* larvae. For chemical screening the methanolic crude extracts obtained from the selected strains were analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC-UV/RI). About 30 actinomycete strains were isolated and were identified as different species of genus *Streptomyces*. Among all the selected strains, the isolates M72, M1, W38, W101 and M93 were found to exhibit potent antimicrobial activity against the test strains including *Klebsiella*, methicillin resistant *Staphylococcus aureus* (MRSA), *E.coli*, and *B.Cereus*. Similarly the isolates M54, W19 and M81 demonstrated significant cytotoxicity (up to 80% larval mortality) against *Artemia salina*. The study suggested that actinomycetes flora of Chenab River is rich source of useful natural compounds and should be continuously isolated characterized and investigated for novel antibiotics and other chemotherapeutic agents.

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**DESERT ACTINOMYCETES: ACTIVE AGAINST METHICILLIN RESISTANT  
*STAPHYLOCOCCUS AUREUS* (MRSA), ANTIMICROBIAL SCREENING, METABOLIC  
FINGERPRINTING AND 16S RRNA GENE SEQUENCING**

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The changing pattern of diseases and the emergence of resistant bacterial strains against currently used antibiotics continuously put demand for novel antibiotics. Actinomycetes is the most important group of bacteria for the production of medically valuable secondary metabolites specially antibiotics. The aim of this study is to isolate and screen the desert actinomycetes against methicillin resistant *Staphylococcus aureus* (MRSA). In this context 80 actinomycetes strains were selectively isolated from the soil and sand samples of Cholistan desert, in southern Punjab, Pakistan. These strains were identified by morphological, biochemical, physiological characterization and by 16S rRNA gene sequencing. The isolates exhibited promising antimicrobial activity against MRSA and in some cases significant zone of inhibition up to 20 mm or even more were recorded in agar diffusion and disc diffusion assays against MRSA. The selected isolates were cultivated as 1-2 liters shaking cultures and in lab fermenters and subsequently the active compounds were extracted and were characterized by thin layer chromatography (TLC), HPLC-UV/RI and HPLC-MS. The activity against MRSA and production of diverse metabolites as depicted by chemical profiling reveal that these desert actinomycetes are the proliferant producers of useful antimicrobial agents, and should be probed further for novel/potentially useful drugs against MRSA.

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**ANTIMICROBIAL EFFECT OF SOME MEDICINAL PLANT EXTRACTS AGAINST  
ORAL BIOFILMS**

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This study aims at checking the inhibitory effects of different plant extracts on biofilm forming microorganisms isolated from clinical setting. A total of 60 samples including 30 from oral sites and 30 from urine and wounds were collected and 50 morphologically different strains were isolated. Six highly resistant strains were characterized morphologically, physiologically, biochemically and genetically. Isolated strains were tested for biofilm formation using test tube assay, congo red assay and liquid-interface coverslip assay. Antibacterial activity of aqueous and methanolic extracts of 5 different plants including *Camellia sinensis* (green tea), *Syzygium aromaticum* (clove), *Musa sapientum* (banana), *Mentha piperita* (peppermint) and *Allium sativum* (Garlic) was determined both individually and in combination against selected strains in both planktonic and biofilm mode. 16srRNA sequencing identified strains as *Providencia stuartii*, *Shigella sonnei*, *Escherichia coli*, *Bacillus cereus*, *Enterobacter aerogenes* and *Macroccoccus caseolyticus*. Significant biofilm formation was observed by each of the three methods for all strains except for *E. coli* and *P. stuartii*. Aqueous extract of *A. sativum* showed highest antibacterial activity against all tested strains with MIC ranging from 75-735 mg ml<sup>-1</sup> and MBC from 255-740 mg ml<sup>-1</sup>. Aqueous extracts of *M. sapientum* exhibited maximum biofilm reduction in *B. cereus*. Reported knowledge of medicinal plants as antibacterial and antibiofilm agents against both highly contagious and antibiotic resistant gram positive and the gram negative isolates provide novel

information necessary to control their formation in clinical setting. Hence, there is an increasing need to research new substances with the ability to inhibit these strains..

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**CLONING, EXPRESSION OF A THERMOSTABLE LACCASES FROM  
*ANOXYBACILLUS RUPIENSIS* AND ITS ROLE IN DECOLOURIZATION OF  
INDUSTRIAL WASTEWATER**

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Thermophilic bacterial strain *Anoxybacillus rupiensis* have ability to detoxify industrial waste containing azo dyes or textile fabric dyes. The strain was optimized and was confirmed phylogenetically and morphologically. Methyl violet, textile industrial waste from one of the textile mills of Faisalabad, market fabric dyes and dyed fabric cloth were decolourized with the help of laccase enzyme secreted by *A.rupiensis*. Decolourization was solely due to enzyme and not due to physical adsorption of dyes on microbial cells surfaces because on centrifugation of dye decolourizing bacterial culture the obtained pellet was totally colorless. Maximum decolourization of 62% was observed at 60 °C when dye solution (filtered effluent) inoculated with bacterial culture was incubated for 5 days. Inducers such as guaiacol and CuSO<sub>4</sub> were added in media containing bacterial cultures to enhance laccase production. Laccase presence was confirmed by using syringaldazine as a substrate and development of purplish color was observed. 1.5 kb lac gene was amplified from *A.rupiensis*, cloned into the cloning vector (pTZ57R/T) and confirmed by sequencing. Lac gene was ligated in expression vector pET-21a and transformed in *E. coli* BL21 codon plus. Expression of un-induced and IPTG-induced laccase was observed on SDS-PAGE. The protein band of laccase was found to be of 55.5 kDa.

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**PREDOMINANT PATHOGENS ASSOCIATED WITH NOSOCOMIAL INFECTIONS IN  
HOSPITALIZED PATIENTS**

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Patients hospitalized in ICUs are 5 to 10 times more likely to acquire nosocomial infections than other hospital patients. The frequency of infections at different anatomic sites and the risk of infection vary by the type of ICU, and the frequency of specific pathogens varies by infection site. Contributing to the seriousness of nosocomial infections, especially in ICUs, is the increasing incidence of infections caused by antibiotic-resistant pathogens. Prevention and control strategies have focused on *methicillin-resistant Staphylococcus aureus*, *vancomycin-resistant Enterococcus*, and *extended-spectrum  $\beta$ -lactamase-producing Gram-negative bacilli*, among others. Studies have shown that patients infected with resistant strains of bacteria are more likely than control patients to have received prior antimicrobials, and hospital areas that have the highest prevalence of resistance also have the highest rates of antibiotic use. The most frequent types of hospital-acquired infection: pneumonia, surgical site infection (SSI), urinary tract infection (UTI), and bloodstream infection (BSI).

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## 5. MOLECULAR BIOLOGY

### MOLECULAR DETECTION AND RISK FACTORS OF HUMAN T-LYMPHOTROPIC VIRUS IN THALASSEMIA PATIENTS IN KHYBER PAKHTUNKHWA, PAKISTAN

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Human T-lymphotropic virus (HTLV) modify human CD4+ and CD8+ T-lymphocytes causing lifelong persistent infection. HTLV-I and HTLV-II cause an aggressive malignancy (adult T-cell leukemia) and neurodegenerative condition respectively. Blood transfusion (whole blood, platelets and packed red blood cells), is the main route of transmission and people (like thalassemia patients) receiving blood and blood products are at great risk of acquiring HTLV. This study is designed to identify the presence of HTLV in thalassemia patients of Khyber Pakhtunkhwa. 343 thalassemia patients (registered in blood providing centers of Peshawar) were analyzed for HTLV through PCR. Demographic and clinical data and risk factors of infection were collected through a predesigned questionnaires. Of the total beta thalassemia patients included in this study, 196(57.1%) were male and 147(42.2%) were female with mean age of  $9.59 \pm 4.57$  (2-25) years. Only 2 (0.58%) samples were confirmed positive for HTLV-I while HTLV-II was not detected in any of the patient. Both the individual positive for HTLV were female [1.3% (2/147)], in age groups 10-15 years [0.9% (1/108)] and >15 years [2.3% (1/43)]. Risk factors observed were family background (satisfactory/poor family 2/210=0.95%), general surgery (dental surgery, splenectomy 1/31=3.22%, circumcision in male) and ear/nose piercing (2/130=1.53%) in female. Presence of HTLV in this specific blood receiving patients shows a threat of this virus in general population. HTLV should be included in routine screening of infectious agents during blood transfusion in Pakistan.

### MUTATIONAL ANALYSIS OF AN OIL DEGRADING GENE FROM LOCALLY ISOLATED BACTERIAL STRAINS

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Petroleum hydrocarbons are one of the most important contaminants in our environment, as they cause damages to the surrounding environment like in sea water and oil refineries. Removal of these contaminants is becoming an immense problem. The current study aimed to isolate an oil-degrading bacterial strain. From a number of isolate only 2 isolates showed positive results for oil degradation. These two isolates were then mutated randomly to observe the effect of mutation on enzyme activity. Irradiated bacterial colonies showed marked decrease in C23O enzyme activity, compared to their wild-type isolates. Using gene specific primers, the gene for key hydrocarbon degrading enzyme *i.e.* Catechol 2, 3 Dioxygenase (C23O) was amplified in both wild type and mutant strains. Random Amplified Polymorphic DNA (RAPD) analysis demonstrated genome

differences and various polymorphic bands between the wild-type and their UV irradiated mutant isolates. These mutated isolate's gene products could be sequenced for further study.

#### **CHARACTERIZATION OF MOLECULAR MARKERS FOR IDENTIFICATION OF *SALMONELLA ENTERICA* SEROVAR TYPHI**

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Typhoid is a highly dangerous disease caused by *Salmonella enterica* serovar typhi and it prevails in less advance areas. However, if it remains untreated then it might lead to gastrointestinal bleeding, intestinal perforation and in severe cases it can be fatal. Over 20 million deaths are annually reported to be caused by typhoid all around the world. Being systemic and infectious this disease causes approximately 90% of deaths in Asia. Typhoid is often not detected through diagnostic tests such as Widal test, typhidot etc. which in many cases also lead to death of patients. The case control study was carried out by employing 58 typhoid patients and 70 healthy individuals in Pakistani people. Two specific primer pairs were used to amplify target genes of Flagellin (498 bp) and StyR-36 (204 bp) of *Salmonella enterica* serovar Typhi. The data obtained was then statistically analyzed. Current study has revealed that PCR sensitivity of flagellin gene is 77.58% and styR-36 gene is 68.97%, clearly showing the fact that these primers are sensitive. However, the specificity of these primers is 100% as no control sample showed PCR result. Therefore, these primers can be used for the diagnostic purposes and are more sensitive as compared to Widal test and typhidot etc.

#### **ASSOCIATION OF THE eNOS G894T POLYMORPHISM AND THE RISK OF MYOCARDIAL INFARCTION IN THE PAKISTANI POPULATION**

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Diabetes mellitus (DM), is the condition which involved group of metabolic disorders, which are responsible for hyperglycemia. Number of genetic and lifestyle factors are found to be associated with the onset and course of the disease. Here we have focused on gene of one of the important enzyme; endothelial nitric oxide synthase (eNOS), involved in the production of nitric oxide (NO), which found to play important role in metabolic homeostasis. The change in single nucleotide polymorphism (SNP) in eNOS gene reportedly found to associate with DM patients. Many studies confirmed the association of different polymorphic sites in eNOS gene with DM. One of the polymorphic site; T786C in 5' flanking region of eNOS gene, is one of important site found to be associated with DM in different ethnic groups and populations. The case control study was carried out by employing 100 DM patients and 100 age and sex matched healthy individuals. The specific primer pair was used to amplify 248 bp eNOS gene fragment. RFLP with *Ban II* enzyme was used to detect the polymorphism at G894T site. The data obtained was statistically analyzed for Chi square, ORs and genotypic distribution among cases and control. The genotypic distribution of mutant (GT and TT) in cases is found to be 84%, significantly higher than that in controls 48%.

The p value ( $< 0.01$ ) shows the genotypic frequency of mutant types is significantly higher in patients compared to controls. OR for allelic frequency is 0.27 (95% CI 0.17-0.41), indicating that the polymorphic site is associated with DM and is potential risk factor for Pakistani population.

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**CELL FREE DNA - A NOVEL BIOMARKER IN THE FIELD OF ONCOLOGY: A COMPARATIVE ACCOUNT IN THE CANCER PATIENTS AND HEALTHY RESIDENTS OF KARACHI CITY**

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Circulating DNA or cell free DNA (cf-DNA) is created during cell death and various malignancies in the body. This is named so because it contains DNA fragments that are present outside the nucleus, circulating freely in the blood stream. The most important factors which determine the levels of cf-DNA are age, tumor volume, tumor histology, lymph node metastasis and tumor progression. The present study was aimed to ascertain the levels of cell free DNA (cf-DNA) in the serum samples of diagnosed cancer patients having various malignancies and healthy residents of Karachi City. Collection of blood samples was carried out with informed consent from 62 diagnosed cancer patients and 38 healthy human subjects at various hospitals of Karachi City. Serum was isolated within 2 hours of collection and subsequently about 50  $\mu$ l of each serum sample was analyzed for cf-DNA with genomic DNA kit YGE100, accompanied with Phenol-Chloroform method and was finally processed with Thermo NanoDrop-2000/2000c Spectrophotometer at PCMD, University of Karachi. The present method produced significantly higher yields of cf-DNA. About 50  $\mu$ l of water was added to the isolated DNA pellet and incubated at 60°C for 30-60 min till the DNA pellet was dissolved. An equal amount of Isoamyl Phenol and Chloroform mixture was added to the isolated DNA, vortexed at room temperature for 3-5 minutes and then centrifuged at 3000 rpm for 10 min. About 2  $\mu$ l of the purified cf-DNA fraction of each sample was processed by Thermo NanoDrop-2000/2000c Spectrophotometer. A cf-DNA data file was generated, which showed the amount and purity of cf-DNA in each serum sample. Mean ages of the subjects in the cancer and control groups were 38.5 years and 33.3 years respectively. Positive percentage of cf-DNA was found to be 36.84 % (14/38 samples) in the healthy subjects and 56.45 % (35/62 samples) in the cancer patients. The detected mean level of cf-DNA in the control and cancer group was found to be 1758.8 ng/ $\mu$ l and 5584.2 ng/ $\mu$ l respectively. Minimum and maximum detected levels of cf-DNA in the control and cancer groups were 17.4 - 8757.6 ng/ $\mu$ l and 5381.3 - 18031.5 ng/ $\mu$ l respectively. Significantly elevated levels of cf-DNA were detected in the cancer cases compared with the healthy subjects. Leukemia and lymphoma cases generally were having higher mean levels of cf-DNA compared with other studied types of cancer. Higher serum cf-DNA levels in the cancer cases compared with the healthy subjects is associated with the level of damage caused to nuclear DNA in various malignancies. Blood cancer cases have shown higher concentrations of cf-DNA than other types of tumors. A simple cost-effective blood test for the application of novel cancer biomarker (cf-DNA) will assist clinicians to implement therapeutics in an efficient and effective way for the diagnosis, staging and prognosis of various cancers during cancer management.

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**PHYLOGENETIC ANALYSIS OF *PANTHERA PARDUS* OF PAKISTAN USING COI FOLMER REGION SEQUENCE**

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Mitochondrial genome has been successfully used for phylogenetic studies of different vertebrates. Cytochrome C oxidase subunit I (COI) gene fragment commonly known as DNA barcode have potential to perform taxonomic role in addition, is used to resolve the phylogeny of animal species. Common leopard is one of the important wildlife species inhabiting wide range including Pakistan. Here we used DNA barcoding to identify *Panthera pardus* samples obtained, can be applicable to non-invasive field samples. Moreover the sequence data obtained for COI gene fragment was compared with other sequences of genus *Panthera* in the GENBANK database. The phylogenetic analysis reveals its relation to other species and sub species of the genus. So COI gene is powerful tool to identify the species and phylogenetics of common leopard.

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**MOLECULAR IDENTIFICATION OF MICROFLORA OF *ALECTORIS CHUKAR* EGG SHELLS**

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Chukar partridge (*Alectoris chukar*) is the national bird of Pakistan found across all provinces. Partridges including Chukar partridge (*Alectoris chukar*) are threatened by plethora of problems mainly because of illegal hunting, poaching, habitat destruction and indiscriminate use of pesticides and herbicides. Increased mortality of Chukar partridge embryos during incubation is due to many intrinsic and extrinsic factors includes alteration in albumin ratio, yolk index and temperature variations. Moreover, microbial contamination of eggshells during incubation is known to be a reason of the mortality of embryos. Current study is aimed to isolate the inner shell microflora of *Alectoris chukar*, their molecular identification and its possible effects on the embryo mortality. A total of 204 eggs of *Alectoris chukar* were collected during May to August from Balkasar Research Complex, Chakwal, Pakistan. After completion of incubation period, these samples were divided into 4 categories, *i.e.*, un-pricked with dead embryo, pricked with dead embryo, hatched and unfertilized eggs. 47 out of 204 samples (23.03%) showed bacterial growth. After culturing, DNA was extracted to amplify 16s rRNA gene by using modified universal primers set *i.e.* 8F and 1495R. The results showed the presence of 6 different bacterial strains in the inner eggshells of *Alectoris chukar* which are; *Bacillus thuringiensis* (4.25 %); *B. cereus* (23.40 %); *B. toyonensis* (6.38%); *B. amyloliquefaciens* (19.14%); *B. subtilis* (38.29%); and *B. sciuri* (10.63 %). Finding of the current study led the way to find out the possible effects of microbial load and their metabolites found inside of Chukar partridge (*Alectoris chukar*) eggshell, on embryo mortality as one of the major reason outside controlled environment.

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### GENOTYPIC ANALYSIS AND PHENOTYPIC EFFECT OF MYOD1 GENE ON MEAT QUALITY IN *BOS INDICUS*

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Variations in the quality of meat are because of dissimilarities of heredity and climatic factors that are responsible for controlling and prediction of metabolic processes in myogenesis. Meat production is based on process of the embryonic development and formation of muscle tissue which are under control of MYOD gene family, which regulates the myogenesis during embryonic development and in their differentiation and maturation. The MYOD is a gene family which consists of four genes: MYOD1, MYF4, MYF5 and MYF6 each having (03) Exons and (03) Introns. Myogenin differentiation MYOD1 gene animal. Present research was conducted to identify SNPs in MYOD1 gene and to determine its possible effect of mutations on meat quality traits in 04 Pakistani indigenous cattle namely Red Sindhi Cattle Breeds, Cholistani Cattle Breed, Dhani Cattle Breed and Thari Cattle Breed. SNPs were identified through PCR-SSCP and confirmed through DNA sequencing method. Ten SNPs (4 from EXON-1, 3 from EXON-2, 3 from INTRON-1 region) were identified in MYOD1 gene, whereas 5 of them were missense mutations and 2 were silent mutations and remaining 03 polymorphs of the intron region did not cause any change in the codon sequences. In addition, c.679G>A polymorphism in Exon-2 of MYOD1 gene approached significance difference for both genotypic frequencies (AA and AB Genotypes) as well as Allelic frequencies for A and B allele in all (03) cattle breeds namely Red Sindhi Cattle Breed, Cholistani Cattle breed and Dhani Cattle Breed, whereas highly significant difference was observed ( $P < 0.01$ ) for the Thari Cattle Breed. The results which will show the significant information for betterment of meat quality traits in 04 Pakistani cattle breeds.

### MOLECULAR CHARACTERIZATION OF THE 5'-REGULATORY REGION OF FABP4 GENE IN RED SINDHI CATTLE BREED

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Fatty acid binding protein 4 (FABP4) is a member of the FABP family of proteins that bind fatty acids, which performs multifunctional roles in fatty acid uptake and intracellular transport. In the present study, we cloned the 5'-regulatory region of bovine and identified its transcription initiation sites. Sequence comparative analysis demonstrated amino acids and promoter sequences of were highly conservative in mammals. Real-time PCR analysis revealed the products of bovine were very highly expressed in subcutaneous adipose tissue. Serial deletion constructs of the bovine 5'-regulatory region evaluated in a dual-luciferase reporter assay showed that 209 bp upstream from the transcription initiation site was its core promoter. An electrophoretic mobility shift assay combined with a site-directed mutation experiment indicated that peroxisome proliferator activated

receptor gamma (PPAR $\gamma$ ), CCAAT/enhancer-binding protein  $\beta$  (C/EBP $\beta$ ), and sterol regulatory element-binding protein (SREBP) were important transcription factors for bovine. These results provide an important basis for further understanding the regulation of bovine.

#### MICROSATELLITE DNA POLYMORPHISM OF *CHANNA MARULIUS* INHABITING RIVER JHELM

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*Channa marulius* has great medicinal importance but currently it has been categorized as threatened in Asia. Wild populations of *C. marulius* has undergone a steady decline due to habitat deterioration, over exploitation and aquatic pollution. We analyzed genetic polymorphism by using microsatellite DNA markers in natural populations of *C. marulius*. Samples were collected from different sites of river Jhelum viz. Trimu Headworks, Rasul Barrage, Mangla Dam, Jhelum Bridge and Marala Dhand. A total of five microsatellite loci (CA05, CA07, CA08, CA09 and CA10) were used to evaluate genetic polymorphism. The overall populations of *C. marulius* showed moderate level of heterozygosity. The mean observed heterozygosity ( $H_o$ ) at all the five loci was 0.43 where as expected heterozygosity ( $H_e$ ) was 0.54. The pairwise population genetic differentiation ( $F_{ST}$ ) ranged in between 0.000001 to 0.231955. Analysis of molecular variance (AMOVA) revealed that majority of variations among populations than within populations 62.05% and 33.03%, respectively. Significant deviation ( $p < 0.05$ ) from Hardy-Weinberg equilibrium was seen in all the populations with clear deficit heterozygosity suggesting inbreeding. A UPGMA tree based on Nei's genetic distance matrix revealed that *C. marulius* populations were divided into two major clusters. Principle component analysis showed same clustering of populations as in UPGMA dendrogram on the basis of genetic distance and genetic identity. The present study about genetic polymorphism and population genetic structure of *C. marulius* populations will be fruitful for the standardization of effective management and conservation of fisheries resources.

#### NUCLEOTIDE VARIATIONS IN THE 16S RRNA GENE IN INDO WEST PACIFIC POPULATIONS OF *PORTUNUS SANGUINOLENTUS*

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*Portunus sanguinolentus* commercially important and edible species of Portunid crabs found in coastal waters of Pakistan. During present study Interpopulation and intrapopulation nucleotide sequence variations in mitochondrial DNA (mtDNA) 16S rRNA gene were observed by using Molecular Evolutionary Genetics Analysis (MEGA 6). The 16S rRNA gene was used to amplify DNA from 3 individuals of *P. sanguinolentus*. By sequencing these PCR amplicons and then aligning the nucleotide sequences, it was confirmed that these amplicon aligned specifically to

mtDNA sequences from the similar species of origin from India, China and Vietnams. There were no significant inter and intra-population difference observed in *P. sanguinolentus*, indicated that high gene flow occurred in Indo west Pacific populations.

#### MOLECULAR DEPENDENCE OF GOLD RESISTANCE ON COPPER RESPONSIVE GENES IN *KLEBSIELLA PNEUMONIAE*

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Bacteria use a wide variety of resistance systems to survive in the presence of elevated levels of various metals. Cue and Cus are amongst the main systems that members of enterobacteriaceae use for combating toxic effects of copper and silver. Here, we have shown that these systems are also involved in conferring resistance to gold in *Klebsiella pneumoniae*. The Cue system comprises three genes *cueR*, *copA* and *cueO*. The Cus system is a regulon having two operons: *cusCFBA* and *cusRS*. Expression of *copA*, *cueO*, *cueR*, *cusCFBA* and *cusRS* was quantified at transcriptional level through real time PCR in the presence of non-lethal, sub-lethal and lethal concentrations of gold. These were later compared with the respective mRNA levels in the absence of gold. The results demonstrated that in *K. pneumoniae*, the Cue and Cus systems also play role for resistance against gold. The *cue* determinants showed maximum increase at transcriptional level after induction by gold salt. The increase in *copA*, *cueO* and *cueR* transcripts was 108, 17.8 and 1.58 folds, respectively at 0.05 mM Au<sup>3+</sup> concentration. *cusCFBA* and *cusRS* transcripts showed less fold increase in mRNA in response to gold. Several fold increase in expression of *copA* and *cueO* in *K. pneumoniae* reveals that Cue system is mainly responsible for imparting resistance to gold in this bacteria.

#### DNA HYPOMETHYLATION AND FRAGMENTATION AS A DIAGNOSTIC TOOL IN BREAST CANCER PATIENTS

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This study entitled “DNA analysis: hypomethylation and fragmentation in breast cancer patients of Allied Hospital, Faisalabad” was carried out. The blood samples and information related to patients was collected. The %DNA fragmentation was carried out using diphenylamine (DPA) assay followed by spectroscopy whereas methylation levels were determined using HPLC after enzymatic digestion of DNA. Statistical analysis including mean  $\pm$  S.E., ANOVA and correlation was carried out. It was observed that levels of %DNA fragmentation obtained from cancer patients were significantly higher as compared to controls ( $P < 0.001$ ). Furthermore, %DNA fragmentation was positively correlated with stage of breast cancer ( $P < 0.001$ ), being lowest *i.e.*,  $36.22 \pm 0.88$  (mean  $\pm$  S.E) in stage I and highest *i.e.*,  $57.97 \pm 1.91$  (mean  $\pm$  S.E) in stage III of breast cancer. Age of the patients also had a significant effect on degree of %DNA fragmentation ( $P < 0.001$ ). The results showed that minimum value of fragmentation (mean  $\pm$  S.E) was observed in first age group ( $\leq 30$  years) *i.e.*,  $35.17 \pm 2.36$ , and the highest value (mean  $\pm$  S.E) of  $65.42 \pm 1.39$  was observed in last age group (61-70 years). Incidence of breast cancer was slightly higher in left side (58%) but the

site of cancer had no significant effect on DNA fragmentation ( $P>0.05$ ). Similarly family history had non-significant effect on DNA fragmentation ( $P>0.05$ ). Percentage DNA fragmentation was positively correlated with age of the patient, stage of breast cancer, tumor size and duration of disease ( $P<0.001$ ). These findings suggests that DNA damage increases with the stage of cancer as well as duration of malignancy. It was observed that methylation levels were significantly lower in cases as compared to controls ( $P<0.001$ ). Methylation levels were correlated with stage of the cancer and age of the patients. Being lowest in stage III (mean $\pm$ S.E. =  $2.227\pm0.07$ ) and highest in stage I of breast cancer (mean $\pm$ S.E. =  $3.38\pm0.10$ ). Methylation levels were lowest (mean $\pm$ S.E. =  $1.94\pm0.12$ ) in last age group and highest in first age group (mean $\pm$ S.E. =  $3.61\pm0.19$ ). There was no difference in methylation levels with respect to family history and site of breast cancer ( $P>0.05$ ). These findings suggest that DNA profiles from peripheral leukocytes can be used as a bimolecular marker to diagnose and manage breast and other malignancies.

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**MISSENSE MUTATION IN EXON 2 OF GLUCAGON LIKE PEPTIDE-1 RECEPTOR GENE AND RISK OF DEVELOPING DIABETES MELLITUS TYPE 2"BACKGROUND**

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Diabetes mellitus is a heterogeneous syndrome of metabolism, characterized primarily by hyperglycemia. It is caused due to defects in insulin release, its action or both. Diabetes mellitus type 2 is the most common form of diabetes with 90-95% prevalence. This study is aimed to examine that whether Arg44His; CGC to CAC polymorphism in exon 2 of the GLP-1R gene is associated with current study type 2 diabetes patients of Lahore or not. Mutational screening for Exon 2 of GLP-1R gene of type 2 diabetes patients and healthy individuals was done by PCR-RFLP analysis using Hin1I restriction endonuclease. 30 T2DM subjects and 20 controls were analyzed. The results showed that CGC to CAC, Arg44His polymorphism was absent in all the T2DM patients and the healthy individuals. Thus it is safely concluded that the polymorphism R44H in exon 2 of GLP-1R gene is not the cause of T2DM in the current study subjects of Lahore population.

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## 6. PHYSIOLOGY

### ROLE OF INSULIN LIKE GROWTH FACTOR-1 IN ISCHEMIC HEART DISEASE IN OUR LOCAL POPULATION

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Ischemic heart disease (IHD) as one of cardiovascular disease becomes a major public health concern worldwide. Insulin-like growth factor-1 (IGF-1) is a protein and prominent biomarker of IHD risk. The aim of this study was to evaluate the relationship of IGF-1 with IHD and its potential risk factor. For this purpose cross-sectional study was done on 96 subjects. All the subjects were divided into two groups as IHD group (n=71) and control group (n=25). IHD subjects were taken from Punjab Institute of Cardiology Lahore, Pakistan. Control group subjects were enrolled from normal healthy population. Biochemical parameters such as lipid profile were assessed by using chemistry analyzer. ELISA was performed for the cardiac enzyme (CK-MB) and hormonal assessment (IGF-1) in the laboratory of LCWU. Statistical analysis was done by using SPSS version 16.0 (ILO, Chicago). High %age of smoking, diabetes, family history and low %age of physical activity was found out in IHD, Total Cholesterol, CK-MB and IGF-1 were significantly higher while triglycerides and body mass index were non-significantly higher in IHD group as compared with control group. The relationship of IGF-1 with TG and CK-MB was direct while relationship of IGF-1 with total cholesterol was inverse. It is concluded that IGF-1 is an important risk factor for the progression of IHD and it is related with several other risk factors of IHD.

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### PREVALENCE OF ANTERIOR CORNEAL DYSTROPHIES IN PAKISTANI POPULATION

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Present retrospective, analytical study was performed from November 2014 to June 2015 on all cases seen from different cities of Punjab during ophthalmological consultation at various hospitals of Lahore including Layton Rehmatullah Benevolent Trust hospital, Mughal Eye hospital, General hospital and Mayo Hospital. The aim of this research was to determine the prevalence of Anterior Corneal Dystrophies (ACDs) in Pakistani population. For the diagnosis of Anterior Corneal Dystrophies, techniques were used for examination of the eye and inspection of cornea using slit lamp microscopy, topography, far visual acuity, keratometry and pachymetry. Drop in the visual acuity and accumulations of material in the cornea were the main reasons for this consultation. 52 cases of Anterior Corneal Dystrophies (ACDs) were identified out of 9000 cases which correspond to 0.5 % hospital prevalence. Out of the 52 cases, 21 were female and 31 were male patients. Anterior Corneal Dystrophies were predominant in the age groups 15-45 years. Visual disability is the most common complaint recorded from the patients of Anterior Corneal Dystrophy. Among the 52 cases, three types of ACDs were reported. Epithelial Basement Membrane (EBMD) is the most prevalent type recorded in our cases. Two other types Meesmann (MECD) and Reis-Buckler (RBCD) are rare and few cases were recorded of them. The diagnosis of

ACDs is usually later in life. The understanding of each type of ACDs is needed to identify genetic mutations and their causes responsible for it. Genotyping of the effected families should be performed to obtain a linkage pattern of this disease and its various types. Clinical trials approved by the government and new possible therapies should be introduced along with intervention of the patients to effectively control ACDs.

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#### **RELATIONSHIP OF VITAMIN D WITH SEVERITY OF KIDNEY DISEASE IN OUR LOCAL POPULATION**

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The present study was aimed to assess the relationship of vitamin D with risk factors of chronic kidney disease. CKD is a progressive loss in renal function over a period of months or years. High Prevalence of CKD in Pakistani population is due to hypertension and diabetes. In Pakistan, number of patients with chronic Kidney disease is increasing persistently. Approximately 20 million adults in the United States are in various stages of CKD. The risk factors for chronic kidney disease include an age more than 60 years, hypertension, diabetes, cardiovascular disease and a family history of CKD. Vitamin D deficiency is found in the majority of patients with chronic kidney disease (CKD). A prospective cross sectional study was conducted in Services hospital, Lahore from January to June, 2014. Data was collected from Nephrology wards and out-door patients departments. All the patients were belonged to 40 to 80 years. CKD was diagnosed on the basis of estimated Glomerulus Filtration Rate (eGFR). This study involved 17 healthy subjects (Control) and 75 patients of CKD with stages 1-5. Their demographic profiles were recorded and blood samples taken for serum vitamin D, parathyroid hormone, Creatinine, Urea, Uric acid, Calcium, Albumin and Phosphate assessment. Measurement of biochemical parameters was done on chemistry analyzer, while vitamin D and PTH by ELISA on CODA. Pearson's correlation was used to find the relationship between the studied parameters. All statistical analysis was done on SPSS 16.0 version. Age and BMI showed significant difference ( $p \leq 0.05$ ) increase in CKD group as compared to control group. There was also a highly significant increase ( $p \leq 0.01$ ) in serum creatinine, urea, uric acid, albumin and calcium in CKD group as compared to control group whereas significant ( $p \leq 0.05$ ) reduction of serum phosphate occurred in CKD group due to increase in PTH. Hormonal analysis revealed that the level of vitamin D decreased significantly ( $p \leq 0.05$ ) in CKD group as compared to control group and PTH significantly ( $p \leq 0.05$ ) increased in CKD group. The comparison of biochemical parameters (serum creatinine, uric acid and phosphate) among different stages of CKD showed significant ( $p \leq 0.05$ ) increased in CKD patients while serum urea, albumin, calcium increases non-significantly. Hormonal analysis of Vitamin D and PTH among different stages of CKD was highly significant ( $p \leq 0.01$ ). The present study has shown a high prevalence of Vitamin D deficiency and insufficiency in a group of patients with CKD. The serum level of Vitamin D in CKD group was  $15.64 \pm 0.53$  ng/ml and in control group, it was  $38.14 \pm 3.90$  ng/ml with highly significant difference between the groups ( $p \leq 0.01$ ). The patients with CKD have less exposure to sunlight. Prevalence of CKD is a gender-dependent. This disease is more common in females as compared to males. Vitamin D is significantly decreased in CKD patients. Impaired vitamin D metabolism in CKD including reduced vitamin D synthesis in the skin and urinary loss of vitamin D metabolites causes deficiency of vitamin D which may exert adverse consequences on renal structure and function.

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**ASSOCIATION OF BREAST CANCER WITH THYROID DISORDERS**RABIA SHAHEEN<sup>1</sup>, MUHAMMAD RAFI<sup>2</sup>, ABIDA RAZA<sup>3</sup> AND NASEEM IRFAN<sup>1</sup><sup>1</sup>*Pakistan Institute of Engineering and Applied Sciences, Nuclear Engineering Department, Nilore, Islamabad, Pakistan*<sup>2</sup>*Pakistan Institute of Engineering and Applied Sciences, Physics and Applied Mathematics Department, Nilore, Islamabad, Pakistan*<sup>3</sup>*Nuclear Medicine, Oncology and Radiotherapy Institute, Molecular Diagnostic Department, Islamabad, Pakistan*

The association between breast cancer and thyroid disorders is controversial. Discrepant results have been reported in the literature. The incidences of thyroid diseases were investigated in patients with breast cancer and control individuals without breast or thyroid disease. Clinical and ultrasound evaluation of thyroid gland, determination of serum thyroid hormone and antibody levels, and fine-needle aspiration of thyroid gland were performed in 80 breast cancer patients and 16 control individuals. The urinary iodide levels of 100 breast cancer patients and 60 controls have also been determined. The mean values for anti-thyroid peroxidase and anti-thyroglobulin antibodies were significantly higher in breast cancer patients than in control individuals ( $P = 0.0345$  and  $0.0451$ ). The incidences of thyroid nodules were higher in breast cancer patients than in control individuals (67.5% versus 6.25%,  $P = 0.000$ ). The urinary iodide levels of breast cancer patients were lower than the controls ( $P = 0.0326$ ). The mean values for free thyroxine was lower and thyroid stimulating hormone was higher in breast cancer patients than in controls ( $P = 0.049$  and  $0.035$ ) respectively. Our results indicate an increased prevalence of thyroid diseases in breast cancer patients.

**STUDY ON RISKS ASSOCIATED WITH GESTATIONAL DIABETES MELLITUS IN WOMEN VISITING DIFFERENT HOSPITALS OF LAHORE**

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The study was performed to determine the risk factors of gestational diabetes in women. The study was conducted on 250 subjects between the age of 21-35 years which included 50 non-pregnant, 50 normal and pregnant and 150 gestational diabetics. Demographic measurements such as age, height, weight, blood pressure, family history of diabetes and eating habits. Blood samples were collected and transferred to laboratory to analyze the glucose levels. Analysis of variance (ANOVA) was applied on the parameters for the comparison of mean between groups. The socioeconomic status of subjects was low. With increasing age the risk of diabetes also increases. The gestational diabetics have family history of diabetes. The mean age in gestational diabetics was  $28.15 \pm 0.38$  years while in non-pregnant and pregnant was  $24.74 \pm 0.53$  years, mean BMI in gestational diabetics was  $29.8 \pm 0.48$  kg/m<sup>2</sup> while in non-pregnant and pregnant was  $25.43 \pm 0.72$  kg/m<sup>2</sup>, mean of systolic blood pressure in gestational diabetics was  $135.53 \pm 1.02$  mmHg while in non-pregnant and pregnant was  $119.40 \pm 0.72$  mmHg. The diastolic blood pressure in gestational diabetics was  $93.13 \pm 0.82$  while in non-pregnant and pregnant was  $80.00 \pm 0.00$  mmHg and  $75 \pm 0.71$  mmHg respectively. The mean glucose level in gestational diabetics was  $224.8 \pm 6.95$  mg/dl while in non-pregnant and pregnant was  $73.24 \pm 2.26$  mg/dl and  $83.00 \pm 1.45$  mg/dl respectively. The



mean HbA1c value in gestational diabetics was  $11.77 \pm 4.63$  mmol while in non-pregnant and normal and pregnant was  $4.19 \pm 0.08$  mmol and  $4.56 \pm 0.05$  mmol respectively. The age, BMI, blood pressure, family history, glucose level and HbA1c value are high in gestational diabetics, as they were at a higher risk of developing gestational diabetes.

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#### PROTECTIVE EFFECT OF RUTIN AGAINST CISPLATIN INDUCED REPRODUCTIVE TOXICITY IN ADULT MALE RATS

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The present study aimed to find the protective effects of rutin against Cisplatin (CP) induced testicular toxicity. Twenty adult male rats were divided into 4 groups. First group was given saline orally while second group received intra-peritoneal (i.p) injection of CP (7mg/kg) on day first. Third group received i.p injection of CP at day one and treated with rutin (75mg/kg) orally for next 12 days. Fourth group was treated with rutin orally for 13 days. Animals were sacrificed on 14th day. CP treatment resulted in a significant decrease in DSP. DNA damage showed significant increase in number of comets/120 cells, tail length, % DNA in tail and tail moment while significant decrease in head length and % DNA in head. CP treatment also caused adverse morphological and histopathological changes in testis of rat, including reduced epithelial height, tubular diameter and increased luminal diameter. Number of spermatogonia, primary and secondary spermatocytes and spermatids were significantly reduced. Additionally, CP treatment also resulted in a significant increase in the thiobarbituric acid reactive substances (TBARS), whereas other antioxidant enzymes were significantly decreased in testicular tissues. Rutin co-treatment resulted in reversing cisplatin effect on DNA damage, sperm count, histological and biochemical parameters. Similarly, cisplatin treatment significantly changed the intratesticular testosterone concentrations while plasma testosterone concentrations were not different statistically. Restored intratesticular testosterone concentrations were observed in rutin co-treated animals. Our results indicated that rutin co-treatment provide protection against cisplatin induced reproductive toxicity in male rats.

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#### EFFECT OF VITAMIN E INCREMENT IN FEED ON GROWTH AND ANTIOXIDANT ENZYMES PROFILE OF *Oreochromis niloticus*

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In a feeding trial of sixty days, impact of vitamin E was studied on the growth and antioxidant enzyme profile of *Oreochromis niloticus*. Vitamin E was mixed @ 0(VE00), 100(VE100) and 200(VE200) mg/kg of the diet and fed to stock of fish separately. The feed was given daily @ 7% of body weight. Parameters viz. body weight and total length were measured

after every week as measure of growth. At the end of experiment fish species was dissected and sample of gills, liver and meat were taken to analyze the CAT and SOD and the meat samples for body composition respectively. *Oreochromis niloticus* showed highest growth in VE100 as compared to the other groups. The group fed with less than 100 mg/kg vitamin E showed less growth and enzyme activity. As the vitamin E increased in the feed the activity of CAT and SOD increased in liver and gills of *Oreochromis niloticus*. From the present experiment it was concluded that vitamin E had an upright role in growth and defense action of *Oreochromis niloticus*.

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#### **AMELIORATIVE EFFECTS OF RUTIN AGAINST METABOLIC, BIOCHEMICAL AND HORMONAL DISTURBANCES IN POLYCYSTIC OVARY SYNDROME IN RATS**

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The study was commenced to study the effects of Rutin on metabolic, biochemical, histological, and androgenic aspects of polycystic ovary syndrome in rats. PCOS were induced by the oral administration of Letrozole (1 mg/kg) in six weeks old female Sprague Dawley rats for 36 days. The control group received 0.5 % aqueous solution of carboxy methyl cellulose (CMC) for 36 days. PCOS, Metformin, Rutin-I & II groups received Letrozole (1mg/kg) throughout the experiment and Metformin (2mg/100g), Rutin (100mg/kg, 150mg/kg) post treatment was given to last three groups from day 21 to day 36 respectively. On day 37, animals were euthanized, and ovaries were taken out. Anthropometrical, metabolic and histological analysis was carried out. Biochemical and androgenic profiles were also evaluated. Both the doses of Rutin significantly reduced oxidative and androgenic levels. A complimentary lipid profile, CRP value and a decrease in the proportion of estrus smears were observed in treatment groups. Histopathological examination of ovary revealed a significant decrease in thickness and number of cystic follicles in post treated groups. The study provides an evidence for the potential ameliorative effects of Rutin in PCOS.

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#### **TARGETING INSULIN RESISTANCE THROUGH GABAERGIC MECHANISM; POSSIBLE CURATIVE EFFECT OF GABA IN LETROZOLE INDUCED PCOS IN RATS**

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Present study was designed to investigate the possible protective and ameliorating effects of GABA in Letrozole induced PCOS model in rats by targeting insulin resistance. Adult female rats (n=25), 200±15 were divided into five groups according to the treatment. Group 1 was taken as control and was given 0.5 % CMC through gastric incubation, group 2 was allocated as PCOS group and was given 1mg/kg/day of letrozole dissolved in 0.5%CMC, group 3 was allocated as co-treated group and was given Metformin (2mg/kg), group 4 was treated with Letrozole (1mg/kg/day) and GABA (100mg/kg/day) and group 5 received Letrozole (1mg/kg/day) and GABA (500mg/kg/day). All the animals were euthanized on the 37 day and blood samples were collected

and plasma was separated and stored at -20°C. Ovaries were removed, weighed and left ovary was placed at -80°C for the analysis of antioxidants while right ovary was washed with saline and placed in 10% formalin for histological processing. Antioxidants such as catalase (CAT), superoxide dismutase (SOD) and peroxidase were significantly reduced in PCOs as compared to the control, while CAT, SOD, POD significantly increased by metformin, GABA 1 (100mg/kg) and GABA 2 (500mg/kg). TBARS and ROS significantly increased in PCOS treated group as compared to the control group. Testosterone (T) levels significantly decreased in GABA 2 treated group while Estradiol (E<sub>2</sub>) concentration significantly increased in GABA 2 treated group. Histological studies showed that the number of cystic and atretic follicles were significantly high in PCOs group. The results suggest that GABA treatment has shown protective effect in PCOs model of rats and provide beneficial effect either by reducing insulin resistance or by inducing antioxidant defence mechanisms. That can in turn provide a picture of possible treatment of PCOS by oral administration of GABA and ameliorating effect of GABA against insulin resistance in PCOS.

#### STUDIES ON SEMEN MORPHOLOGY AND EFFECTS OF ARTIFICIAL INSEMINATION ON HATCHABILITY IN TURKEYS (*MELEAGRIS GALLOPAVO*)

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This preliminary study was carried out with turkey (*Meleagris gallopavo*) by collecting semen from 4 male turkeys and insemination into 6 hens. Semen was collected from male turkeys by abdominal massage and pooled once a week for 6 weeks. It was evaluated based on volume, sperm concentration, live-dead-ratio and mass motility which were; 0.19±0.04mls (confined male) and 0.19±0.029mls (Free range male), semen concentration 6.35±0.87 (×10<sup>9</sup> cell/ml) and 5.33±0.54 (×10<sup>9</sup> cell/ml), live-dead ratio 69.25±14.97 and 71.66±11.72 and motility 73.01±8.47 and 81.41±7.19 for confined and free range reared turkeys. The semen samples were examined morphologically and mean abnormal rates and types were evaluated. A total abnormal spermatozoa rate of 14.61±1.61% was determined. Most common defects were acrosome's defects which determined as 36.67±3.80% and second most common defects determined in mid-piece of sperms as 8.59±0.24. Head defects leading defects up to 7.15±1.21% of total defects of semen morphology. Tail defects of 6.69±1.97% were the next most prevalent. Hens were inseminated weekly. Laid Eggs were collected, stored and later incubated for hatching. Forty (40) eggs were collected from females which were naturally inseminated shows 86.67%, 81.82% and 78.57% hatchability, similarly artificial inseminated eggs shows 87.50%, 93.33% and 85.71% hatchability. From the result it was clear that semen collected from the turkeys (Toms) rose at Turkey's research and Breeding Center at UVAS was of satisfactory quality and can be used fresh in artificial insemination. The result also signifies satisfactory hatchability of eggs from inseminated hens. In conclusion, we observed that the most common morphological defects were at the acrosome and the mid-piece, and these organelles were the most sensitive to various environmental conditions.

**EFFECT OF FERRIC SULPHATE ON THE HAEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILE OF *CTENOPHARYNGODON IDELLA***

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Present study was conducted to report the effect of different concentrations (47.5, 50.5, 55.5 and 58.5 mgL<sup>-1</sup>; T<sub>1</sub>-T<sub>4</sub> respectively) of ferric sulphate, Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, on complete blood count and selected serum biochemical parameters of grass carp (*Ctenopharyngodon idella*) upon four day exposure. *C.idella* with average body length 14.7 ± 2.37 cm and average body weight 35.92 ± 17.7 g were distributed into five groups. A common untreated control group was maintained in parallel. Blood samples from all treatments were collected by direct cardiac puncture at the end of the experiment. Our results indicated an increased glucose concentration in T<sub>1</sub> (P = 0.001), T<sub>2</sub> (P = 0.048) and in T<sub>3</sub> (P = 0.016) as compared to control group indicating stress response in *C. idella*. An increase in platelets count in T<sub>3</sub> (P = 0.018) and decreased MCH (P = 0.02) and MCV (P = 0.033) in T<sub>3</sub> were observed indicating abnormal CBC upon Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> exposure. Serum analysis revealed a significant (P=0.02) decrease in total protein concentration in all Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> treatments. ALT in T<sub>3</sub> (P=0.01) and AST in T<sub>4</sub> (0.002) were significantly higher than control indicating abnormal liver physiology. These results suggest that ferric sulphate had moderately deleterious effects on *C. idella* but further research is needed to explore its impact on gene expression profile.

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**MEASUREMENT OF LEVELS OF ETHINYLESTRADIOL AND TESTOSTERONE IN AGRICULTURAL AND PHARMACEUTICAL WASTE WATER IN LAHORE; EFFECTS OF THESE ENDOCRINE DISRUPTOR ON GONADAL DEVELOPMENT IN *LABEO ROHITA***

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This study measured the levels of ethinylestradiol (EE<sub>2</sub>) and testosterone (T) hormones released into environment through effluent water of pharmaceutical and agricultural industries in Lahore, Pakistan. A total of 36 drain water samples were collected from pharmaceutical, agricultural, and municipal wastewaters. Level of hormones in water samples were measured by using ELISA. Significant values (P<0.05) of hormones have been found in all samples. The effects of EE<sub>2</sub> and T as endocrine disruptor on gonadal development in juvenile *Labeo rohita* EE<sub>2</sub> and T released in sewage water from industries are finally released in irrigation water stream or seeps through soil to reach underground water. Utilization of this contaminated water in fish farms may seriously disrupt their gonadal development. To investigate the effect of these endocrine disruptors, Fish were kept in aquaria at the ambient water temperature and photoperiod. One control (n= 10) and two treatment groups (n=7 in each treatment group) were studied for the period of 30 days. Treatment groups were exposed to the dose of 5µg/L of EE<sub>2</sub> and T on daily basis, post trial histological examination of gonads showed a few primordial germ cells and spermatogonia in control group. Sterility was observed in all fish treated with hormones. This study observed the high levels of T and EE<sub>2</sub> in pharmaceutical, agricultural and municipal wastewater and their negative effects on gonadal development in *Labeo rohita* in Lahore, Pakistan.

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**ASSESSMENT OF DIFFERENT OILS SOURCES AS ENERGY REQUIREMENT AND THEIR IMPACT ON GROWTH PERFORMANCE OF JUVENILE BLACK FIN SEA BREAM, *ACANTHOPAGRUS BERDA* (FORSSKAL 1775)**

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In this study, effect of different oils on growth, nutrient utilization and body composition of juvenile blackfin sea bream *Acanthopagrus berda* was investigated. Fish juvenile were collected from Sonari channel, Hawksbay, Karachi and were brought to the aquaculture laboratory. After acclimatization of 15 days they were randomly distributed in rectangular tanks (Size 3ft x 1.5 ft x 1.5ft each). In each tank 10 fish were stocked, with three replications for each treatment. Four isonitrogenous diets with different oils sources (fish oil (FO), soybean oil (SO) olive oil (OO) and palm oil PM) were fed to juvenile of sea bream *A. berda* for 60 days. Best specific growth rate (SGR) and feed conversion ratio (FCR) were noted in the fish fed with fish oil (FO). Secondly, best growth enhancement was found in fish fed with soybean oil (SO) while fish fed with Olive oil (OO) and Palm oil (PM) showed poor SGR and FCR value in contrast of fish oil (FO). Body composition was not significantly influenced by lipid source although low crude lipid was found in fish fed with FO. The hepatosomatic index (HSI) and viserosomatic index (VSI) was greater in FO than the remaining diet. Finally, it was concluded that fish oil (FO) is the best source of energy in fish diet followed by soybean oil (SO) for *A. berda* weighing from 10.1 g and 69.2 g. Further study is required for optimization of fish oil and replacement of fish oil (FO) with vegetative oil sources.

**FATTY ACID ANALYSIS OF FISH SILAGE PREPARED BY DIFFERENT METHODS AND EFFECT OF FISH SILAGE ON FISH DIGESTIVE SYSTEM ENZYMES.**

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Present study was conducted to analyse fatty acid profile of fish silage prepared by acid and fermented method and possible effect of diets prepared by incorporation of different ratio of fish silage on activity of fish intestine enzymes. Fish acid silage was prepared by mixing of formic acid and fermented fish silage was prepared by mixing Lactobacillus bacteria, molasses and yogurt with minced fish body viscera. Lipids were extracted from pure silage of both types and fatty acid profiles were determined by liquid chromatography. Result showed that both types of silage contain large amount of unsaturated fatty acids. Further three treatment diets of each silages were prepared containing 100% silage, 75% silage and 50% silage and fourth treatment was prepared by fish meal instead of fish silage. Prepared treatment diets were fed to *Labeo rohita* fingerlings @ 4% of fish body weight twice a day. At the end of 90 days feeding trial the fish were dissected and their intestine were homogenized and analyzed for enzymes *i.e.* protease, amylase and lipase activity. The enzymes activity showed that protease activity non significantly vary according to protein

concentration while lipase and amylase activity vary according to lipid concentration and carbohydrate concentrations, respectively.

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**TITANIUM DIOXIDE NANOPARTICLES INDUCED DNA DAMAGE, RENAL TOXICITY AND HEMATOLOGICAL ALTERATIONS IN MALE SPRAGUE DAWLEY RATS**

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Titanium dioxide (TiO<sub>2</sub>) is one of the most commonly used materials and is among the top five nanoparticles. Due to the extensive application of TiO<sub>2</sub> nanoparticles in the industrial field and on-going commercialization of nanotechnology products, the exposure of the human body intentionally or unintentionally to nanoparticles via several possible routes, including dermal penetration, inhalation, oral ingestion or intravenous injection is possible and may continue to increase, so an evaluation of their potential toxicity is essential. This study investigated the toxic effects of TiO<sub>2</sub> nanoparticles on kidney and blood of male Sprague Dawley rats. Rats were injected subcutaneously with TiO<sub>2</sub> nanoparticles (NPs) at four different dose levels of either control (0) or 50 or 100 or 150 mg/kg of body weight of rat. Animal mortality, haematology, micronuclei assay and kidney histology were investigated at the start of experiment and 28 days after treatment. After twenty eight days of exposure dose dependent toxicity was observed in different treatment groups. At the end of treatment the micronuclei frequency increased significantly in rats treated with the highest (150 mg/kg) dose of TiO<sub>2</sub> nanoparticles (P<0.05). TiO<sub>2</sub> nanoparticles exposed groups showed significant dose dependent pathological variations in kidney such as reduction of renal glomerulus number, apoptosis or vacuolization, infiltration of inflammatory cells, tissue necrosis or disorganization of the renal tubules, and dilation in tubules. However, the blood cell count changed significantly in all treated groups (P<0.05) as compared to control groups.

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**RELATIONSHIP BETWEEN SERUM RESISTIN AND INDICES OF OBESITY IN YOUNG PAKISTANI SUBJECTS**

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Obesity is currently regarded as a low grade chronic proinflammatory condition that affect the metabolic homeostasis. Correlations between inflammatory and metabolic cells are the major inflammatory inputs that leads to obesity related disorders. Resistin acquired initial attention as a potential link between obesity and glucose regulation, while its implication in the control of insulin sensitivity is still a matter of debate in humans. The aim of this study was to investigate the relationship between serum resistin levels and metabolic parameters in obese Pakistani subjects. Study subjects included 300 over weight, obese males and females with an age ranging from 17 to 30 years. 100 comparable control subjects were included. The data was stratified on the basis of BMI into normal weight, overweight, Obese I and obese II following the WHO criteria for Asians. Anthropometric parameters including age, BMI, waist circumference, WHR, systolic and diastolic

blood pressure were assessed. Serum Resistin and insulin levels were measured by ELISA. Lipid profile and fasting glucose were measured using chemistry analyser. Insulin sensitivity was calculated by fasting insulin. Serum levels of resistin and insulin, fasting glucose, triglycerides and cholesterol were significantly high in overweight and obese subjects as compared to normal weight subjects ( $p < 0.01$ ). In obese group, Resistin showed a significant positive correlation with BMI, fasting glucose, systolic B.P and Diastolic B.P ( $r = 0.352$ ,  $p < 0.01$ ;  $r = 0.278$ ,  $p < 0.01$ ;  $r = .279$ ,  $p < 0.01$  and  $r = 0.329$ ,  $p < 0.01$ ) respectively. Resistin levels were significantly associated with cholesterol, insulin levels and insulin sensitivity ( $\mu\text{IU/ml}$ ) ( $r = .375$ ,  $p = .000$ ;  $r = 0.336$ ,  $p < 0.01$ ;  $r = -.231$ ,  $p < 0.05$ ) in overweight group. This study demonstrates a positive correlation between resistin and metabolic markers of obesity in young Pakistani subjects.

#### **EFFECT OF EXOGENOUS ENZYME ON THE NUTRIENT DIGESTIBILITY AND DIGESTIVE ENZYME ACTIVITY IN *LABEOROHITA* FED WITH OIL SEED MEAL BASED DIET**

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Exogenous enzymes in fish feed proved to be important in improvement of growth performance in different animals. Sixiso-nitrogenous (300g protein/kg) and iso-caloric (4000 Kcal/kg) diets with and without enzyme (control) supplementation were fed to *Labeorohita*. 180 fish were randomly assigned to experimental groups. Fish were fed two times per day on floating extruded diet. After 80 days of feeding, nutrient (protein, fat and minerals) digestibility and digestive enzyme activity were checked. Exogenous enzyme showed positive effect on growth and improvement in nutrients digestibility but the activity of digestive enzymes remain unaffected by the addition of exogenous enzyme.

#### **EFFECTS OF HIGH STOCKING DENSITY ON CONDITION FACTOR, LEVELS OF FREE T4 AND CORTISOL IN *CATLA CATLA***

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Aquaculture has major contribution in nutrition, economy and providing healthy food to man all over the world. It is one of the fastest growing food-producing as well as employment sectors in globe. In Pakistan, the welfare of fish is a subject of increasing interest and one of the principal areas of concern is stocking density. The present study aims at assessing effect of stocking density on growth and serum concentration of thyroxin and cortisol in *Catla catla*. For this study, 30 days old, mixed sex fish *Catla catla* ( $n = 28$ ; total body weight = ;  $79.3 \pm 0.43$  total length =  $12.611 \pm 0.204$ , control = 5, treatment = 23) reared for 26 days in tanks with 80 % water exchange, provided oxygen by aerators and fed 2% of body weight twice a day. Treatment groups were introduced with stocking density of 7.9 g/L, while control groups with 4.4 g/L. At the end of trial, fish were killed by overdose of anesthesia (80 g of clove oil per liter). Blood samples were taken to measure the levels of free T4 and cortisol by using ELISA. Condition factor of fish exposed to high stocking density was found significantly low as compared to control groups. After trial, cortisol levels were found to be highly elevated in treatment group ( $425.22 \pm 0.07$  nmol/L) as compared to control group

(87.53±0 nmol/L) after trial however, levels of free T4 were found to be slightly elevated in treatment (1.48 ± 0.18 nmol/L) as compared to control group (0.092±0 nmol/L) after trial. All the results were found to be significantly different from each other ( $P<0.05$ ). Growth suppression and serum hormonal disturbance in treatment groups revealed stocking density has negative effect on growth and welfare of animal. This study recommends that appropriate stocking density for Catla catla should be less than 7.9 kg/L.

### HAEMATOLOGICAL PROFILE OF WILD AND FARMED *CIRRHINUS MRIGALA* (MORI)

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A study was conducted to observe and compare the haematological profile, viz: haemoglobin (Hb), white blood cells, red blood cells, platelet, PCV, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of *Cirrhinus mrigala* either in wild conditions, at Head Trimu and raised in, Fish Seed Hatchery, Satayana Road, Faisalabad. The water quality parameters of both wild and hatchery conditions showed highly significant difference but remained in favorable limits for fish health and survival. Erythrocyte count and Leukocyte count showed highly significant difference in hatchery raised *Cirrhinus mrigala*. Thrombocyte count, haemoglobin, haematocrit and mean corpuscular volume in wild and hatchery raised *Cirrhinus mrigala* differed highly significantly but remained within the favorable limit in both media. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were significant different but remained favorable. Wild fish showed slightly better meat quality in reference to haematological profile than farmed *Cirrhinus mrigala*.

### EFFECT OF CRYOPRESERVATION ON INDIAN RED JUNGLE FOWL (*GALLUS GALLUS MURGHI*) SPERMATOZOA

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The experiment was designed to elucidate the changes in semen characteristics of Indian red jungle fowl spermatozoa during five stages of cryopreservation. Semen from eight mature cocks were pooled, diluted at 37°C in Beltsville poultry semen extender, cooled to 4°C in two hours (37-20°C and 20-4°C/hour), equilibrated for 10 min after addition of 11% glycerol. Cooled semen was filled in 0.5 mL French straws and kept over liquid nitrogen vapours for 10 minutes and plunged into liquid nitrogen. Frozen semen was thawed at 37 °C for 30 seconds. Sperm motility, plasma membrane integrity, livability, and viability were assessed at the stage of pre-dilution (37°C), post-dilution (37°C), cooling (20°C), equilibration (4°C) and thawing (37°C). Sperm motility did not change at post-dilution and cooling and averaged 83.3 ± 3.1%. However, motility declined



( $P < 0.05$ ) to  $66.0 \pm 2.4\%$  and  $45.0 \pm 6.5\%$  at post-equilibration and thawing stages. The sperm plasma membrane integrity at pre-dilution stage was  $74.8 \pm 1.4\%$  that decreased ( $P < 0.05$ ) to  $62.3 \pm 1.7\%$ ,  $51.7 \pm 1.7\%$ , and  $42.0 \pm 1.2\%$  at the cryopreservation stages of post-dilution, cooling and post-thawing, respectively. A decline ( $P < 0.05$ ) in acrosomal integrity was observed at post-cooling ( $83.8 \pm 2.4\%$ ), equilibration ( $80.8 \pm 0.8\%$ ) and thawing ( $73.8 \pm 2.4\%$ ). It is concluded that maximal damage occurs to plasma membrane and acrosome followed by motility of Indian red jungle fowl spermatozoa at post-cooling, equilibration and thawing stages of cryopreservation. This is the first ever report on the successful cryopreservation of Indian red jungle fowl spermatozoa.

**ISOLATION, PURIFICATION AND PARTIAL CHARACTERIZATION OF  
SUPEROXIDE DISMUTASE FROM THE KIDNEY OF SILVER CARP  
(*HYPOPHthalmichthys molitrix*)**

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Reactive oxygen species (ROS) cause oxidative stress in all aerobic living organisms. Superoxide dismutase (SOD) is a part of antioxidant system and minimizes the damaging effect of free oxidative radicals by converting the superoxide into  $O_2$  and  $H_2O_2$ . In this research work, the purification and partial characterization of superoxide dismutase (SOD) enzyme from the kidney of silver carp (*Hypophthalmichthys molitrix*) was studied. Fish were separated into two groups. One group of fish was kept under chronic exposure of a mixture of metals (Pb+Cr) and other group was present in unstressed conditions. The activity of purified enzyme (SOD) was decreased in kidney of stressed fish as compared to unstressed fish. Enzyme characterization showed that SOD had wide range of pH from 4 to 8.5. Maximum enzyme activity was  $458 \text{ U mL}^{-1}$  for unstressed fish at pH 7.5, however, for stressed fish it was remained as  $570 \text{ U mL}^{-1}$  at pH 6.5. At  $40^\circ\text{C}$  and  $50^\circ\text{C}$  the activity of SOD was increased for unstressed and stressed *H. molitrix* i.e.  $525 \text{ U mL}^{-1}$  and  $550 \text{ U mL}^{-1}$ , respectively. The enzyme activity was also altered at different enzyme substrate (NBT) concentrations for unstressed and stressed *H. molitrix*.

**A STUDY ON EPIDEMIOLOGY AND GENETICS OF MIGRAINE IN PAKISTANI  
SUBJECTS**

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Migraine is a neurovascular disorder characterized by recurrent unilateral headaches accompanied by nausea, vomiting, photophobia and phonophobia. Genetically, migraine is a complex familial disorder in which the severity and the susceptibility of individuals are most likely governed by several genes that vary between families. A random cross sectional study was conducted on randomly selected areas of Pakistan. The study population included individuals of all age groups: 6-100 years. Individuals less than <14 years were self-interviewed and then their performances were filled. For data collection, a self-administered questionnaire was designed

according to ICHD-2 (“International Classification of headache Disorder edition 2”, 2004). Univariate and multivariate logistic regression analyses were performed to compute the crude odds ratios (OR) and adjusted ORs with 95% confidence intervals (CI) for approximation of relative risk associated with experience of migraine, hypertension type headache and other headache. Significance level was  $p < 0.05$ . DNA was subjected to Tetra primer ARMS-PCR to investigate rs1835740 SNP, occurring on 8q22.1 chromosomal region. rs1835740 is situated between the plasma glutamate carboxypeptidase (*PGCP*) and astrocyte elevated gene 1 (*MTDH/AEG-1*) which are involved in glutamate metabolism in CNS. Sex prevalence of migraine was found to be 2:1 (female to male ratio) for gender while high prevalence of migraine was associated with single marital status as compared to married one. There was more representation of the migraine patients from KPK. Male respondents suffering from heart and respiratory disorders, showed association with migraine as compared to females. Males showed association of nocturnal sweating with migraine while females did not show such symptoms. Recent investigations showed that mouth dryness was related to migraine in males as compared to females. In skip meals variable, migraine attacks were frequently prevalent. Both the genders showed strong association between day sleepiness and migraine in the current survey. Digestion problem in migraine patients showed significant association towards migraine in both genders. In present findings symptoms of migraine attack shown by migraineurs included photophobia, phonophobia, osmophobia, anxiety or depression, nausea, vomiting, anorexia, constipation, diarrhea and aura etc. Results of molecular genetic analyses revealed that rs1835740 is the mutation of C/T. Allele C is the ancestral one and its frequency significantly rises in migraine patients along with the rise in the frequency of heterozygotes CT, while in controls subjects the frequency of allele C was higher as compared to allele T while the frequency of heterozygotes CT was lower. In migraine patients, the frequency of allele C was even higher while the frequency of allele T was even lower. The frequency of heterozygotes increased significantly in migraine patients as compared to controls as well. Further in depth investigations are required to be done at national level to study the major SNPs and their association with the disease.

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#### **NIGELLA SATIVA OIL IS AN EFFECTIVE TREATMENT AGAINST ISOLATION-INDUCED AGGRESSION: A STUDY IN MALE LABORATORY RATS**

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Aggression is a reaction that conveys harmful stimuli to other organisms. Psychiatric problems like schizophrenia, bipolar and personality disorders are identified as impulsive aggressive behaviors. For such disorders, the search for identification and characterization of antipsychotics drugs and herbal compounds as remedial alternatives has progressed very rapidly. The effects of distinctive plant extracts on aggressive behavior have been explored studies on *Nigella sativa* are lacking. *Nigella sativa* is an indigenous herb with a wide range of pharmacological potential. The most essential active compound is thymoquinone. Presently, we used isolation-induced aggressive behavior in rodent model to investigate the effect of *Nigella* oil on aggressive behavioral deficits indicating intra-specific aggression. Doses of *Nigella sativa* oil (1 ml/kg and 2 ml/kg) were administered via intragastric gavage to isolated male laboratory rats (n=5 in each group plus controls) for 30 consecutive days. These laboratory rats were then used as “residents”, whereas wild rats were used as “intruders” against these residents. Aggressive behavior

was assessed through isolation-induced aggression paradigm. Behavioral tests were accomplished in the early hours of dark phase. After the last dose of *Nigella sativa* oil, each isolated laboratory rat was confronted with wild rat intruder for a period of 10 min in its home cage. Behavior of rats was recorded with a video camera. The videos were analyzed and scored manually. The latency to first attack, time duration and frequency of offensive aggression were recorded in the resident rats. In addition, latency, time duration and frequency of other non-aggressive behaviors were also measured. Kruskal-Wallis non-parametric ANOVA (one way analysis of variance), followed by Students Neuman-Keuls post hoc analyses were applied to analyze behavioral data. Treatment with 1 ml/kg and 2 ml/kg *Nigella sativa* oil led to a significant increase in the attack latency of isolated resident rats as compared to control group. However, non-significant change was found in the latency period of other behaviors of all treatment groups. Time duration and frequency of attack behavior and offensive behavior in resident rats of both treatment groups reduced significantly ( $P < 0.001$ ) as compared to control rats. In contrast, non-significant change was observed in time duration and frequency of other non-aggressive behavioral categories of both treatment groups when compared with control animals. The present study demonstrated that oil of *Nigella sativa* possesses the potential of anti-aggressive drug and can be used as a prospective medicine for the treatment of aggression condition in humans as well. Further experiments are required to explore this potential before human trials could be done.

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#### EFFECT OF ANTIOXIDANT VITAMINS E AND C ON ESSENTIAL TRACE ELEMENTS OF SELECTED BODY TISSUES OF LABORATORY RATS

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Vitamin C and E are two well-known anti-oxidant nutrients which dispose scavenge and suppress the formation of free radicals and intercept their activities. Vitamin E protects against lipid per-oxidation and prevents specific diseases associated with free radical damage such as cardiovascular disease, chronic inflammation and cancer. Whereas vitamin C is essential for normal growth and physiological functions in animals and supports spermatogenesis in testis. The present study was conducted to investigate the effect of antioxidant vitamins E and C and their combination on trace element concentration in male and female laboratory rats. Rats were divided into four groups *i.e.* I (saline), II (treated with 300 mg/kg b.w. Vitamin E), III (treated with 500 mg/kg b.w. vitamin C) and IV (treated with 150 mg/kg b.w. vitamin E along with 300 mg/kg b.w. vitamin C). Female rats showed higher levels of Cr, Zn, Ni and Co concentration in whole blood as compared to male rats. Similarly, Fe and Cu concentration in the whole blood of females were decreased as compared to males. Furthermore, the concentration of Cr and Fe was decreased in male rat brain, while no significant change in Cr concentration was observed in female rat brain but Fe level was significantly increased after treatment with vitamin E and C. Co concentration was increased in female rat brain upon treatment with vitamin E and C while it showed no significant change in male rat brain. Moreover, Mn concentration was increased in brain of both sexes. In seminal vesicle, Zn concentration was increased while Cr, Fe, and Ni concentration were not altered after treatment. Where as in testis, Fe and Ni levels were increased, Pb was decreased and cobalt concentration was not altered after treatment. The study concludes that the administration of vitamin E and C leads to modulation of Cr, Fe, Ni, Co, Cu, Mn and Ni concentrations of male and female rats.

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**STUDY OF DIETARY VITAMIN E REQUIREMENT OF MORI, *CIRRHINUS MRIGALA* FINGERLINGS**

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Present project was intended to study the dietary vitamin E requirement of mori, *Cirrhinus mrigala* fingerlings. Fingerlings were kept in V-shaped tanks with stocking density of 15 fish/tank with an initial average weight of  $2.78 \pm 0.01$  g. Six experimental diets were formulated based on graded levels of vitamin E i.e. 0, 25, 50, 75, 100 and 125 mg/kg of diet. Each treatment was applied to two replicates and fish were fed once daily at 2% live wet weight for a feeding period of 2 months. Fish were weighed after each week in order to determine the growth performance. At the end of the feeding experiment, five fish from each replicate were collected and sacrificed and liver, gills and muscle samples were extracted to determine the thiobarbituric acid reactive substances (TBARS) content, antioxidant enzymes (i.e. superoxide dismutase, peroxidase and catalase) and fatty acid profile, respectively. Growth responses in terms of final weight, absolute weight gain, weight gain% and specific growth were significantly ( $p < 0.05$ ) higher in diet containing 100 mg/kg vitamin E. Similarly, improved feed conversion ratio (FCR) and feed intake (FI) were observed at the same level of supplementation. Groups fed with high levels of dietary vitamin E showed significant ( $p < 0.05$ ) decline in values of TBARS concentration. A significant ( $p < 0.05$ ) increase was detected in the antioxidant enzyme activities with increased vitamin E supplementation. Dietary vitamin E supplementation showed significant ( $p < 0.05$ ) effect on different fatty acids when compared with control group. Maximum values of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), n-7 and n-9 fatty acids were recorded in control group, while polyunsaturated fatty acids (PUFAs), n-3 and n-6 fatty acids were found maximum in vitamin E supplemented diet in muscles of *C. mrigala* fingerlings. In conclusion, the results of the present study suggested that the dietary vitamin E supplementation positively influence the growth performance, antioxidant status and fatty acid profile. As a proficient antioxidant, vitamin E has potential to reduce oxidative stress in *C. mrigala* fingerlings.

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**EFFECTS OF TANNERY WASTEWATER ON MICRO- AND MACRO ELEMENTS CONCENTRATION AND HISTOMORPHOLOGICAL FEATURES OF LIVER AND KIDNEY TISSUES OF FISH *CHANNA PUNCTATA* FROM SIALKOT-WAZIRABAD**

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Leather tanning is one of the major industrial sectors in Pakistan. It contributes a large share of foreign exchange but at the expense of severe environmental pollution hazardous to humans, animal and plant life forms. The present study assessed the impact of tannery wastewater containing salts and heavy metals on a fish (*Channa punctata*) normally inhabiting nullahs that receive industrial refuse. The study was undertaken in Sialkot-Wazirabad cities and suburbs. Metal

concentrations were determined through atomic absorption spectrophotometry. Histomorphological damage to the liver and kidney tissues was assessed. Biochemical estimation of serum levels of ALT, ALP and bilirubin was also carried out. Comparisons were made statistically. In liver, significantly elevated concentrations were found for some macro- and microelements: Na ( $p < 0.05$ ), Ca ( $p < 0.01$ ), Mg ( $p < 0.001$ ), Cr and Fe ( $p < 0.001$ ), Cu, Pb and Zn ( $p < 0.001$ ). The order of heavy metals concentration in polluted water fish was: Fe > Zn > Cr > Ni > Pb > Cu > Co > Cd. In the kidney tissue significantly increased concentrations were observed for: Na ( $p < 0.01$ ), Ca ( $p < 0.01$ ), Mg ( $p < 0.001$ ), Cr ( $p < 0.001$ ), Cu, Fe, Pb, Zn and Ni ( $p < 0.01$ ). The sequence of heavy metals in the kidney tissue of polluted water fish was: Fe > Zn > Ni > Cr > Pb > Co > Cu > Cd. In the brain tissue following were found raised: Ca ( $p < 0.05$ ), Cr, Cu and Pb ( $P < 0.05$ ), Fe and Zn ( $p < 0.01$ ). Microelements were in the order of Fe > Zn > Ni > Pb > Cr > Cu > Cd. Histomorphopathological abnormalities in the liver of polluted water fish included: pyknotic and irregular shaped nuclei, swollen hepatocytes, increase sinusoidal spaces, necrosis and cytoplasmic degeneration. In the kidney, renal tubules and glomeruli showed degeneration and hypertrophy of tubular epithelial cells, occlusion of tubular lumen, glomerular expansion and shrinkage. Bowman's space was also found increased. The present finding revealed that tannery wastewater profoundly affects cellular and physiological alterations in fish tissues

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**EFFECT OF A BROAD SPECTRUM ANTIBIOTIC CIPROFLOXACIN ON  
REPRODUCTIVE ORGANS, GONADAL STEROIDS AND LIVER FUNCTION IN  
LAYERS OF WHITE LEGHORN BREED CHICKEN**

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Ciprofloxacin is a second generation fluorinated quinolone with very broad antimicrobial spectrum. It is used primarily for the treatment and prevention of coli septicemia, one of the most important bacterial infections in poultry. The present study was carried out to determine the effects of ciprofloxacin on the serum profile of estradiol, progesterone, testosterone and ovarian function in layer pullets of white leghorn breed. A total of fifteen layer pullets of approximately same age and weight were obtained. Flocks were divided into three groups. Group I and group II received oral doses of ciprofloxacin at a concentration of 10 and 100mg/ kg b.w respectively, daily for 12 days while group III served as control and received 0.9% saline solution orally. Blood samples were collected at day 0, 4, 8 and 12 through brachial vein puncture. Estradiol, progesterone and testosterone levels were estimated using the radioimmunoassay. Liver function tests were carried out on a spectrophotometer. The birds were sacrificed at day 13 and ovaries and oviducts were excised morphological studies. Eggs were also collected on daily basis to determine the egg parameters. Results showed that serum estradiol and progesterone level decreased significantly ( $p < 0.001$ ) in high dose group, while testosterone levels showed non-significant decline. Ovarian weight was reduced in both low and high dose groups ( $p < 0.001$  and  $p < 0.003$  respectively). The number of large yellow follicles was significantly decreased in both treatment groups ( $p < 0.003$  and  $p < 0.03$ ). Liver enzymes ALT, SGPT, AST were significantly elevated indicating the hepatotoxic potential of the drug. The present study suggests that ciprofloxacin causes suppression of serum estradiol and progesterone levels leading possibly to suppress ovarian function in white leghorn layer pullets. Great care, therefore, must be taken with the use of ciprofloxacin in layer pullets regarding its dosage and duration of administration.

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### INFLUENCE OF DIFFERENT FEED INGREDIENTS ON LIPASE ACTIVITY IN *LABEO ROHITA*

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A three month research trial was conducted to observe the effect of different feed ingredients on digestive enzymes lipase activity of *Labeo rohita*. The experiment was conducted in 10 fiber glass tanks, with stocking density of 10 fish per tank. Guar meal, soybean meal, cotton seed meal and canola meal were used as experimental feed ingredients while fish meal was considered as control, each experiment and control feed ingredient was replicated twice. All the feed ingredients were used in mash form. Fish in each tank was fed @ 4% of its wet biomass. At the end of the trial, three fish samples from each of the experimental and control tanks were collected randomly and analyzed for enzymatic activity. For different feed ingredients lipase activity varied significantly ( $P < 0.05$ ) among anterior part, posterior part and complete intestine.

### DETERMINATION OF TRACE ELEMENTS AND MACRONUTRIENTS IN BLOOD AND BODILY TISSUES OF ENROFLOXACIN TREATED BROILER CHICKEN

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The industrialization of poultry production has led to intensive use of antibiotics in feed mixtures for therapeutic purpose and as growth enhancers. Presently, we investigated the potential effects of enrofloxacin, a fluoroquinolone veterinary antibiotic, on essential trace element (Fe, Cu, Zn, Co, Ni and Mn) and macronutrient concentrations (Mg, Ca, Na and K) in body tissues of broiler chickens following orally administered sub-chronic dose. Twenty four 15-day old broiler chickens were divided into four experimental groups. Group I represented control group while groups II, III and IV received daily dose of 10 mg, 50 mg and 100 mg/kg b. wt. respectively for 7 consecutive days. Toward end of experiment, birds were slaughtered and elemental concentrations were determined through flame atomic absorption spectrophotometer (FAAS). Results were compared statistically. While Ni concentration decreased significantly in blood, lung, kidney, and intestine at all three doses of enrofloxacin, chicken treated with same doses showed a substantial decline of Cu concentration in muscle. Additionally, blood and intestinal Zn concentration showed a significant decline, whereas skin and liver Zn concentration increased significantly. Mn and Fe level in liver and Mg in lung showed significant decline. Co concentration showed a significant inclination at all doses, Ca in brain and lung decreased significantly. All doses caused a significant decline in K in muscle and lung. Intestinal Na and Mg level also decreased along with Mg level in gall bladder. Lowest dose of enrofloxacin (10 mg/kg) increased Mn and Na in skin, Fe and Na level

in pancreas, while decreasing Ca levels were found in pancreas, gallbladder and intestine. Our results demonstrated that enrofloxacin administration led to marked alteration in essential trace element and macro nutrient concentrations in all vital tissues. The study suggests that optimizing dosage regimens of enrofloxacin is an appropriate strategy to minimize its impact on the distribution of essential trace elements and macro nutrients without impairing the therapeutic efficacy and outcome but still better is to phase out the practice of feeding antibiotics to animals as growth enhancers.

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**MICRO AND MACROELEMENTS CONCENTRATION AND  
HISTOMORPHOLOGICAL STUDY OF FISH (*CHANNA PUNCTATA*) INHABITING  
POLLUTED WATER**

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Leather tanning is one of the rapidly growing industrial sectors in Pakistan, contributing a large share to foreign exchange but at the expense of severe environmental pollution that occur as a result of constant effluents discharge into seasonal nullahs. Since fish is an excellent indicator of contamination of aquatic environment, the present study was designed to access the environmental degradation in relation to heavy metal pollution and its implications on human health in general and on aquatic life in particular. To assess the implication of tannery waste water containing salts and heavy metals on aquatic environment and its inhabitants like the fish, turtles, fish eating birds etc. Fish (*Channa punctata*) presumed to be a hard bottom dweller fish and water samples were collected from selected control and polluted sites of Wazirabad city and suburbs. To link environmental degradation and its impact on aquatic life selected macro- (Na, K, Ca and Mg) and microelements (Cr, Cd, Cu, Fe, Ni, Pb and Zn) concentrations were determined through atomic absorption spectrophotometry in the vital organs of fish samples. Histomorphological damage to the liver and kidney tissues was assessed. Biochemical estimation of serum levels of ALT, ALP and AST was also carried out. Mean concentration of different electrolytes and heavy metals were statistically compared. Liver tissue showed significantly elevated concentrations of Na ( $p<0.05$ ), Ca ( $p<0.05$ ), K ( $p<0.05$ ) and Mg ( $p<0.05$ ) and for some microelements like Cr ( $p<0.05$ ), Cd ( $p<0.05$ ), Cu ( $p<0.05$ ), Fe ( $p<0.05$ ), Ni ( $p<0.05$ ), Pb ( $p<0.05$ ) and Zn ( $p<0.05$ ). In the kidney tissue significantly elevated concentrations were noticeable for Ca ( $p<0.05$ ), Cr ( $p<0.05$ ), Cu ( $p<0.05$ ), Ni ( $p<0.05$ ), and Zn ( $p<0.05$ ). For stomach tissue, non-significant differences were found in the concentration of macroelements between polluted and control water fish. For micronutrients significantly increased concentration were found for Cr ( $p<0.05$ ) and Ni ( $p<0.05$ ). Intestine tissue showed significantly elevated concentrations for Na ( $p<0.05$ ), Ca ( $p<0.05$ ) and Mg ( $p<0.05$ ). Among microelements, only Zn ( $p<0.05$ ) concentrations were elevated. For muscle tissue, non-significant difference was found in the concentration of macroelements between polluted and control water fish. For micronutrients significantly increased concentration was found for Ni ( $p<0.05$ ). Significant differences were found between control and experimental fish as regards ALT ( $p<0.01$ ), ALP ( $p<0.02$ ) and AST ( $p<0.002$ ) concentrations. Histomorphological abnormalities observed in the liver of polluted water fish included pyknotic and irregular shaped nuclei, swollen hepatocytes, increased sinusoidal space and cytoplasmic degeneration. In the kidney of fish from polluted sites renal tubules and glomeruli showed marked alterations. Mainly degeneration and hypertrophy of tubular epithelial cells, occlusion of tubular lumen and glomerular expansion was

found in some section while shrinkage was present in others. Bowman's space was also found increased. The present findings clearly revealed the devastating effects of tannery waste water on the physiology of fish under investigation resulting in possible health hazards to humans through food chains.

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#### **EFFECT OF CORIANDER EXTRACT ON REPRODUCTIVE SYSTEM IN MALE LABORATORY RATS**

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Coriander (*Coriandrum sativum* L.) belongs to the family of Apiceae. Coriander is indigenous to the Mediterranean region and is widely cultivated in Russia, Central Europe, North Africa and Asia. Young leaves of the plant are used to make sauces and chutneys. The green leaves are consumed as fresh herbs, in salads and as garnishes due to its attractive green color and aroma. World Health Organization (WHO) estimates that approximately 80 % population of the developing world uses traditional medicine for primary healthcare. However, there is a prevalent misunderstanding that herbal medicines are devoid of toxic effects. Adverse effects of herbs have been reported that include allergic reactions, hepatotoxicity, nephrotoxicity, cardiac toxicity, neurotoxicity and even deaths have been reported. Some adverse effects of coriander on implantation have been reported. Data as regards its effects on male reproduction are lacking. This hypothesis was tested presently. Healthy young male rats were divided into three groups: Group\_I (Control distilled water treated); Group\_II (2000 mg/kg b.w. ethanolic extract of *Coriander sativum* leaves and stem); Group\_III (4000 mg/kg b.w. ethanolic extract of coriander). Pre and post treatment blood samples were for estimation of serum testosterone levels. Male reproductive organs were dissected out for histological studies. Right epididymes were minced and supernatants were taken for sperm count, daily sperm production. Results showed significant decreases in serum testosterone levels and sperm count ( $P < 0.001$ ) as compared to control. Histological results revealed that, in testes basement membrane and tunica albuginae were distorted, lumens of testes were enlarged, leydig cells were irregular and distorted. The present study reveals that excessive intake of coriander may lead to reproductive dysfunction but the effect of coriander as an infertility herb remains to be investigated.

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#### **CORRELATION BETWEEN IRON AND CHOLESTEROL IN GALLSTONE PATIENTS AT HYDERABAD AND ADJOINING AREAS**

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To explore the correlation between iron and cholesterol in gallstone patients at Hyderabad and adjoining areas. One hundred eleven (n=111) gallstone patients treated at different hospitals of Hyderabad and adjoining areas were recruited for present study. Their pre-operated blood samples and post-operated gallstone samples were collected during the year 2014. Gallstone samples were analyzed for their composition by FTIR. All blood and gallstones samples were analyzed for iron



and cholesterol concentrations. Iron concentration in serum as well as in gallstone samples was determined by Atomic Absorption Spectroscopy and serum cholesterol concentration was determined by kit method, whereas, cholesterol in gallstones was determined by FTIR. The correlation of iron and cholesterol in serum and gallstone samples was computed by SPSS version 22. A high level of positive correlation ( $r=0.57$ ) was found between iron and cholesterol in serum, whereas low level of positive correlation ( $r=0.21$ ) in stone samples of gallstone patients. Interestingly, there was a high level of negative correlation ( $r=-0.75$ ) between serum cholesterol and gallstone cholesterol concentrations in patients. But we did not find any correlation between serum and gallstone concentrations for iron. Mid level of positive correlation was seen in serum of cholesterol and mixed stone formers ( $r=0.44$  and  $0.39$  respectively), whereas, high level of positive correlation ( $r=0.75$ ) was found between serum iron and cholesterol concentrations in pigmented stones formers. Low level of correlation was found in all type of stone samples between iron and cholesterol concentrations. High level of positive correlation was also found in both genders between serum iron and cholesterol concentrations. There is a high level of positive correlation ( $r=0.57$ ) between iron and cholesterol in serum, whereas low level of positive correlation ( $r=0.21$ ) in stone samples of gallstone patients.

#### STUDY THE EFFECT OF METALS ON FEMALES INFERTILITY

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Infertility is a disease of reproductive system defined by the failure to achieve clinical pregnancy after 12 months of marriage. Pakistan is among the currently most populous countries of the world, and has a population growth rate of around 2%, it also has high rate of infertility (21.9%); 3.5% primary and 18.4% secondary. This signifies that more than one fifth of the country's married population is directly associated with this problem. However, no investigation of effect of metals on Females' Reproductive System is reported yet. We, therefore, undertake the present study to explore the role of serum metal contents in Infertility. Total 60 confirmed infertile females were randomly selected from infertility center of Hyderabad and 47 age and gender matched controls having negative personal or family history of infertility with 3 or more than 3 biological children. Blood samples were collected from all the selected females and analyzed for serum metal contents by Atomic Absorption Spectrometer. We significantly decreased serum concentration of Zinc, Copper in infertile females as compared to fertile females, whereas, Cadmium was significantly increased in Infertile females. The cadmium analysis according to normal and abnormal hormones revealed significant variation for Luteinizing hormone, whereas Zinc with Anti Mullerin hormone. The copper analysis according to normal and abnormal hormones revealed significant variation for TSH, Prolactin, and Rubella virus. Age wise comparison of infertile females for all metals revealed significantly increased concentration of Cd in 36-45 age group, whereas, Cu show significantly increased concentration of Cu in fertile females of same age group. The Zinc concentration was significantly increased in infertile females with history of miscarriage. We found significant increased level of Cu in Infertile females using Antidepressant drugs. We explored significantly high concentration of Cd in infertile females who used ground water for drinking, whereas, Zn was significantly high in Fertile Females who used Surface water for drinking. We noted significant increased concentration of Cu in infertile females

with high tea consumption as well. In conclusion, serum metal contents (Zn, Cu, and Cd) may play a role in altering the female reproduction which may result in infertility.

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#### **ASSOCIATION OF MENOPAUSAL STATUS WITH GALLSTONE DISEASE IN FEMALE PATIENTS AT HYDERABAD AND ADJOINING AREAS**

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Gallstone disease is a globally accepted health issue in humans. In Pakistan; gallstone disease is increasing day by day. Gallstones in females have been found in all menopausal stages (Pre-, Peri-, Menopause and post- menopause), hence the present study was undertaken to evaluate the association of menopausal status in females with gallstone disease. A total number of 135 female gallstone patients admitted in Liaquat University Hospital, Jamshoro, Wali Bhai Rajputana Hospital, Hyderabad and other hospitals of Hyderabad and 170 controls (menopausal status matched *i.e.* pre-menopausal, peri-menopausal, menopausal and post-menopausal) were selected for the present study. All the patients and controls were interviewed by a self structured standard questionnaire especially designed to study the association of menopausal status with gallstone disease. Gallstones were also recovered from gallstone patients at the time of cholecystectomy and analyzed for composition by Fourier Transform Infrared Spectroscopy (FTIR). We found that majority of gallstone patients were of pre-menopausal status as compared to other menopausal status. The demographic characteristics of gallstone patients revealed that majority (92.6%) females were married, having >3 children, house wives with sindhi ethnic group and residing at Hyderabad, Fried food is significantly positively associated with gallstones disease. Especially fried potatoes are the significant risk factors for this disease. The association of type of oil consumption revealed significant positive association of khula pakwan with gallstones disease. Khula ghee was also significantly positively associated with gallstone disease. As far as monthly consumption of oil or ghee is concerned we explored significant variation between patients and control group. Majority of the female gallstone patients were in the pre- and post-menopausal status. Gallstone composition by FTIR revealed that the majority of gallstones were of pure cholesterol recovered from females of all menopausal status. Mixed composition of gallstones was found in the gallstone recovered from peri- and post-menopausal status. Very few no: of pigmented stones were identified in the pre- and post-menopausal status females. Gallstone composition showed that all gallstones contain 76% cholesterol and 24% bilirubin. In conclusion, we found positive associations of different factors in pre-menopausal status; hence, it may be the associated factor with gallstone disease.

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#### **PRIMITIVE TO PRESENT METHODS OF HUMAN REPRODUCTION IN THE LIGHT OF MODERN SCIENCE**

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Since the primitive age to current age, the humans have been reproduced by both type of reproduction, asexual & sexual via seven different methods. Among these seven methods the three

methods are continued till today and are being recorded, reported and seen practically in this modern age of science. The seven different methods in human being may be listed as below: (1) From no male no female (Pre-existing species of Human Aadam) (2) Regeneration from pre existing species germ material (3) From identical male only, through Natural budding / cloning (4) From identical female only, through parthenogenesis (5) From binary /multiple fission (identical and an identical twins ) (6) From male & female, via fertilization ie. Sexual Reproduction (7) M. No 5 and 6 may appear simultaneously, as reported last year. Among the above seven methods of human reproduction last five methods 3-7 are described scientifically since primitive to modern age. Whereas the last three methods 5, 6 & 7 are continued till today and are seen, recorded and reported practically. Among the three continued method one # 5 can be considered link between asexual & sexual type of reproduction as it is neither strictly asexual and nor strictly sexual. It results from the combination of sexual & asexual type of reproduction. Above all methods except # 1& 7 have been described for animals in science ie Regeneration, Budding / Cloning, Parthenogenesis, Binary / Multiple fission and sexual reproduction but for Humans except 1&2 all other five methods are described in detail scientifically with evidences.

#### **THE PROCESS OF AGING DOES INFLUENCE EGG QUALITY PARAMETERS IN JAPANESE QUAILS**

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This study was conducted at Avian Research and Training Center, University of Veterinary and Animal Sciences Lahore to assess the influence of the process of aging on some egg quality traits in Japanese quails. 24 eggs each at the age of 10, 15, 20 and 25 weeks were subjected to egg quality analysis. Statistical analysis of data using ANOVA procedures under Completely Randomized Design and comparison of means using Duncan's Multiple Range (DMR) Test with the help of SAS (9.1) depicted significant differences in egg weight, egg shape index, yolk index and hough-unit value among the eggs from different age groups. Non-significant differences were observed regarding egg shell thickness.

## 7. TOXICOLOGY

### BIOACCUMULATION AND IMPACT OF HEAVY METALS ON *LABEO ROHITA*

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Heavy metals pollution is the major problem in the aquatic environment. The fishes living in such vicinity have more gambles to become contaminated with metals in their body. In current study concentrations of heavy metals (Pb, Cd, Cr, Zn and Mn) were measured in water and various tissues of *Labeo rohita* (muscle, gills, liver and intestine) collected from Baran Dam and Tanda Dam while using Atomic Absorption Flame Spectrometer. In water of Baran Dam the level of Zn while in Tanda Dam the concentration of Pb and Cr was observed significantly high. Variations in concentration of heavy metals were found in different organs of fish. In muscles the lowest concentrations of metals, while in liver and gills the highest levels were recorded. Heavy metals accumulated in order gills > liver > intestine > muscle in the body of *Labeo rohita* collected from Baran Dam. Similarly, the *Labeo rohita* of Tanda Dam showed the order of metal accumulation liver > gills > intestine > muscle. The order of heavy metals accumulation was found different in both Dams, however in both Dams Pb was the highest and Cd was the least accumulated metals. The concentration of heavy metals in the present study exceeded the permissible levels for water and fish.

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### OCCUPATIONAL EXPOSURE TO CHROMIUM AMONG TANNERY WORKERS IN DISTRICT KASUR, PAKISTAN

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We conducted a survey to assess the health status of tannery workers exposed specifically and/or predominately to chromium. The collected data of the surveyed workers were divided into three age groups (A = ≤ 25, B = 26-39, C = ≥ 40) on the basis of working experience and analyzed statistically. The order of suffering from ailments / disorders of workers belonging to these three groups appeared as C □ B □ A. In overall, the most commonly occurring disorders among tannery workers included skin itching / irritation (90%), dry skin (85%), hypertension (83%), depression (79%), occupational fatigue (74%), dry and productive cough (49%), dizziness (42%), headache (29%), confusion (27%), eyes' irritation (18%), sleeplessness (17%) and sore throat (11%). The overall trend of disorders increased with increase in age and working experience. This alarming situation needs attention of public health welfare authorities.

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### STUDIES ON CHROMIUM BIOACCUMULATION PATTERNS IN INDIAN MAJOR CARPS SAMPLED FROM TRIMMU BARRAGE, PAKISTAN

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Experiment on bioaccumulation of chromium in the bodies of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* was conducted from January through May, 2015. Water, sediment and fish samples were collected on monthly basis from upstream and downstream of Trimmu Barrage. Maximum Cr concentrations from upstream and downstream were recorded as  $23.54 \pm 3.43$  mg/L and  $21.12 \pm 3.47$  mg/L, respectively. Average metal concentration detected from sediment samples was  $71.08 \pm 8.32$  mg/L. Maximum Cr concentration  $81.72 \pm 2.54$  was recorded during February while the same was minimum  $61.24 \pm 1.23$  mg/L during month of May. Among all the fish species, maximum metal concentration was accumulated by *Cirrhinus mrigala* followed by *Catla catla* and *Labeo rohita*. Among fish organs, maximum Cr concentration  $4.75 \pm 0.78$  (µg/g) was recorded in the liver of fish whereas the same was minimum  $1.10 \pm 0.21$  (µg/g) in fish muscles.

### HISTOPATHOLOGICAL ALTERATIONS IN THE GONADS OF PIGEON (*COLUMBA LIVIA*) FOLLOWING EXPOSURE TO PYRETHROID INSECTICIDE-BIFENTHRIN

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Ecotoxicological impact of pyrethroid insecticides are getting attention to their indiscriminate use in agriculture sector. The present study was carried out to observe the histopathological alterations in the gonads of avian species on exposure to bifenthrin insecticide with the aim of establishing their cause of reproductive impairment. A total of 15 pairs (male and female) pigeons (*Columba livia domestica*) equally divided in three groups (A, B and C) comprising 10 birds each. Group B and C pigeons were orally exposed to bifenthrin insecticide on doses  $1/20^{\text{th}}$  &  $1/15^{\text{th}}$  of LD<sub>50</sub> (24mg. and 32mg./kg./day) respectively for 45 days, whereas group A pigeons were kept as control. After exposure to insecticide both test groups birds demonstrated significant decline ( $P < 0.05$ ) and ( $P < 0.01$ ) in their body weight as compared to control group. At the end of experiment birds were sacrificed to dissect out male and female gonads for histological assessments using Haematoxylin and Eosin (H & E) staining techniques. The histopathological alterations in testicular tissues revealed picnosis in the spermatocytes, compressed interstitial space and few of the seminiferous tubules became elongated in the birds of test group B. Group C birds have shown widened interstitial space between seminiferous tubules containing less number of leydig's cells along with hypertrophy. Vacuolation with few spermatocytes were also observed inside seminiferous tubules while in the control group majority of spermatogonic cells were present in the seminiferous tubules. Numerous leydig's cells were observed in the interstitial space. The ovaries in the test group B had partial follicular changes and damaged interfollicular stromal cells. The morphological structure of ovarian follicles was deformed and elongated. In test group C chromatin

material destroyed in the ovarian follicles and zona pellucida was not intact. Necrosis and fibrosis were also observed in the connective tissues and ovarian follicles while histological observations in the control group revealed early and late follicular stages with normal structure of connective tissue. Ovarian follicles had complete nucleus, cytoplasm and granulose membrane (theca interna and externa). This revealed that, exposure to bifenthrin insecticide shown significant histopathological alterations in the male and female gonads of pigeon and this can produce moderate to severe reproductive disorders in the avian species.

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**ASSESSMENT OF ENDOSULFAN EFFECTS ON REPRODUCTIVE AND  
DEVELOPMENTAL PARAMETERS OF MALE FRESHWATER CYPRINID FISH,  
*CYPRINION WATSONI***

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Endosulfan is an organochlorine insecticide. The current study is aimed to assess the effects of endosulfan on reproductive and developmental parameters of male fresh water Cyprinid fish, *Cyprinion watsoni*. The fish was exposed to 0.75 ppb and 1ppb endosulfan on alternate day for 30 days during early spawning season (March). The body length and body weight of 0.75ppb treated group increased significantly ( $p<0.05$ ) compared to control, but with an increased dose of 1ppb body weight showed decrease and the body length increased significantly ( $p<0.05$ ) compared to control. The condition factor (K) and GSI of treated groups showed no change compared to control. Testicular weight, length and breadth of treated fish (0.75ppb) increased significantly ( $p<0.05$ ) compared to control. Negative effects of increased endosulfan dose (1ppb) were observed on testicular weight and breadth, however and increase in the testicular length was examined compared to control. A significant decrease in the mean diameter of spermatogonia was observed in 1ppb group compared to control but not in low dose (0.75ppb) group. Histomorphological studies showed changes *i.e.* loosely arranged lobules, irregular nuclear and cell membrane of spermatogonia, clumping of spermatocytes and spermatids and reduction in sperm count was observed.

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**EVALUATION OF ACUTE TOXICITY OF MANGANESE TO THREE FRESH WATER  
FISH SPECIES**

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The acute toxicity of waterborne manganese (Mn) to the three freshwater fish species viz. *Labeo rohita*, *Cyprinus carpio* and *Oreochromis niloticus* of 120-day age group was evaluated in terms of 96-hr  $LC_{50}$  and lethal concentrations in the wet laboratory under static bioassay at constant pH (7.6), temperature (28°C) and hardness (240mgL<sup>-1</sup>). The mean values of 96-hr  $LC_{50}$  for the fish species were calculated as 74.07, 84.80 and 92.50mgL<sup>-1</sup>, respectively. While their mean lethal concentrations were found to be 124.19, 141.06 and 145.77mgL<sup>-1</sup>, respectively. Regarding overall sensitivity of three fish species, *Labeo rohita* were significantly more sensitive to metal concentrations, followed by that of *Cyprinus carpio* and *Oreochromis niloticus*.

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### QUANTIFICATION AND CLUSTERING OF TERATOGENIC EFFECTS IN ZEBRAFISH LARVAE AFTER EXPOSURE TO TOXICANTS

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The zebrafish larva is increasingly used as an animal model in toxicology and teratology studies because many replicates can be obtained quickly, at low cost, and with relatively small quantities of compound. Previously, we described the population incidence of abnormalities in zebrafish larvae exposed to toxicants. Here, we examine the phenomenon of clustering or co-occurrence of abnormalities in individual larva. A total of 11,214 surviving larvae, exposed continuously from 1 day post fertilisation (dpf) to one of 60 toxic compounds, were scored at 5 dpf for the presence of eight different abnormal phenotypes. These were: pericardial oedema, yolk sac oedema, dispersed melanocytes, bent tail, bent trunk, hypoplasia of Meckel's cartilage, hypoplasia of the branchial arches, and uninflated swim bladder. For 43/60 compounds tested, there was a concentration-dependent increase in the number of abnormalities per larva. In terms of the clustering of abnormalities per larva, yolk sac oedema and dispersed melanocytes were typically the first abnormalities to appear in single larva as the concentration of compound was increased. Pericardial oedema appears a good indicator of teratology. We use the term 'severity score' which is proportional to the number of different abnormalities per larva, and the term 'phenotype cluster' as a qualitative description of which abnormalities are present together in one larva. We developed a metric of teratogenicity ( $TC_{3/8}$ ) which represents the concentration of a compound that produces, on average, 3/8 abnormalities per larva. On this basis, the most teratogenic compounds tested in this study are amitriptyline, chlorpromazine hydrochloride and sodium dodecyl sulphate. The least teratogenic of the compounds tested was ethanol. We find a strong correlation between  $TC_{3/8}$  and  $LC_{50}$  of the 43 compounds that showed teratogenic effects. When we examined the ratio of  $TC_{3/8}$  to  $LC_{50}$ , benserazide hydrochloride, copper (II) nitrate trihydrate and nicotine had the highest specific teratogenicity, while aconitine, hesperidin, and ouabain octahydrate had the lowest. We conclude that these malformations could prove to be general indicators of reproductive toxicity in the Zebrafish embryo assay.

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### CLINCO-HEMATOLOGICAL AND SOME BIOCHEMICAL DISPARITIES INDUCED BY INSECTICIDE FIPRONIL (PHENYLPYRAZOLE) IN FRESH WATER FISH ROHU (*LABEO ROHITA*)

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The aim of this study was to explore clinco-hematological and some biochemical disparities induced by fipronil in fresh water fish rohu (*Labeo rohita*). For this purpose 36 freshwater fishes were purchased from Patiser lake near Lal suhanra National park Bahawalpur and transformed into aquariums having 100 liters of water capacity available in the laboratory of Department of life sciences, The Islamia University Bahawalpur. All the experimental fishes were kept under ideal

conditions in laboratory aquariums. After 2 days of acclimatization all the fishes were randomly divided into 6 groups (6 fishes in each group) named as A, B, C, D, E and F. After grouping fishes were treated with different concentrations of fipronil @ 00, 0.03, 0.06, 0.09, 0.12, 0.15 mg/L for 9 days. The fishes in control group A showed no clinical ailment but other fishes in fipronil treated groups showed increased surface breathing, gasping, jerking, faintness, restlessness and unbalanced body. The fishes treated with higher concentrations of fipronil (0.12 and 0.15 mg/L) showed significant ( $P < 0.05$ ) decrease in erythrocytes, hemoglobin, hematocrit, and monocytes but mean corpuscular volume, mean corpuscular hemoglobin concentration, leukocyte counts, neutrophil, and lymphocyte were significantly increased. Serological parameters ALT, AST, ALP and LDH increased significantly in high dose treated groups hence fipronil induce clinico-hematological and biochemical disparities in fresh water fish *Labeo rohita*

#### ECOLOGICAL RISK ASSESSMENT OF DEET (N, N-Diethyl-Meta-Toluamide) AND ITS BIOLOGICAL USE

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DEET (N, N-Diethyl-Meta-Toluamide OR N, N-Diethyl-3-Methylbenzamide) is widely used as an active broad spectrum ingredient in most of the insect repellents since last five decades. The effect of DEET was studied on kidney cells of monkey to measure its ecological toxicity and the cell survival percentages at different concentrations and  $LC_{50}$  was determined. The different concentrations of DEET were used 0.5 ml, 1ml, 2 ml, 3 ml and 4 ml of DEET in test tubes to take the mean value of optical density 0.1497, 0.354, 0.317, 0.353, 0.454, 0.490, 0.58, 0.400, 0.551 and 0.462 and The cell survival percentages were 4.3%, 50%, 41%, 49%, 72%, 80%, 100%, 60%, 93% and 74%. Results demonstrated that the usage of DEET at CSP (cell survival percentage) 40% is safe for human use and wild mammals as pets to protect them from malarial or dengue attack but percentage of DEET above 40% is not recommended.

#### ASSESSING THE LEVEL OF CATALASE AND METAL BIOACCUMULATION RATE IN THE LIVER AND KIDNEY TISSUES OF WALLAGO ATTU CAPTURED FROM RIVER CHENAB

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The present study was conducted with an objective to measure the level antioxidant enzyme catalase and selected metal including Cr, Cd, Pb, Co and Zn bioaccumulation in the liver and kidney tissues of *Wallago attu* inhabiting in River Chenab. Catalase enzyme is an important part of antioxidant defense system and considered an excellent biomarker for indication of water contamination. For this purpose fish samples were collected from three different stations including Trimmu, Marala and Khanki Headworks of River Chenab. After biopsy, fish liver and kidney were extracted at the sampling station and preserved in an ice box by keeping these organs in



tagged plastic zipper bags for further analyses *i.e.* enzyme assay and metal accumulation rate. The inferences of this study revealed higher hepatic catalase activity in fish captured from Khanki Headworks ( $280 \pm 0.9 \text{ U mL}^{-1}$ ) as compared to Trimmu ( $186 \pm 1.0 \text{ U mL}^{-1}$ ) and Marala Headworks ( $177 \pm 0.8 \text{ U mL}^{-1}$ ). Maximum renal catalase activity was observed in fish collected from Marala Headworks ( $181 \pm 1.0 \text{ U mL}^{-1}$ ) as compared to Trimmu ( $128 \pm 0.9 \text{ U mL}^{-1}$ ) and Khanki Headworks ( $100 \pm 1.0 \text{ U mL}^{-1}$ ). The inferences of present study showed significant difference ( $p \leq 0.05$ ) between Trimmu, Marala and Khanki Headworks *W. attu* liver and kidney catalase enzyme activity. The bioaccumulation rate of metals selected in this study in the liver of fish was recorded as  $\text{Zn} > \text{Cr} > \text{Pb} > \text{Co} > \text{Cd}$  at Trimmu Headworks,  $\text{Zn} > \text{Cr} > \text{Pb} > \text{Co} > \text{Cd}$  at Marala Headworks and  $\text{Zn} > \text{Pb} > \text{Cr} > \text{Co} > \text{Cd}$  at Khanki Headworks while, the deposition rate of these metals in the kidney of *W. attu* were recorded  $\text{Zn} > \text{Cr} > \text{Pb} > \text{Co} > \text{Cd}$  at Trimmu Headworks,  $\text{Zn} > \text{Cr} > \text{Pb} > \text{Co} > \text{Cd}$  at Marala Headworks and  $\text{Pb} > \text{Cr} > \text{Zn} > \text{Co} > \text{Cd}$  at Khanki Headworks. On the basis of present study inferences it is concluded that fish tissues and biomarkers serves as an indicator of metal contamination in aquatic environment.

#### LEAVES OF *WITHANIA SOMNIFERA* L. EXHIBITS PHYTOTOXIC POTENTIAL

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*Withania somnifera*, an herbaceous plant, is known to provide factual evidence for its potential use in traditional medicine systems; but so far, no effort has been made to explore phytotoxic nature of its natural compounds. Hence, to investigate the allelopathic potential of various organic fractions of the plant, a bioactivity, guided study was conducted using *Lactuca sativa* (lettuce) as a test plant. A crude methanolic extract (80%) of its leaves was partitioned into *n*-hexane, ethyl acetate and water fraction successively. Each fraction was monitored against the radicle and hypocotyl growth of the lettuce seedling at various concentration (300, 100, 30, 10, 3, 1, 0.3, and 0.1 mg fresh leaves applied petri dish<sup>-1</sup>) with distilled water as a control. Comparison of their bioactivity revealed that among all sub-fractions, EC<sub>50</sub> value and inhibitory pattern of water fraction was quite similar to that of crude extract, however, the inhibitory activity of ethyl acetate fraction plotted a typical dose response curve. Therefore, the latter was purified by repeated column chromatography, preparative HPLC and analytical HPLC in the last. It resulted in the isolation of two pure allelochemicals identified as Withafirin A and Withanolide D using internal standardization method. The results of the present study depicted that both these products may further be exploited as a potential candidate for the development of bio-herbicides leading to the sustainable agriculture.

**EFFECT OF CADMIUM ON DETOXYFICING ENZYMES OF GRASS CARP  
(CTENOPHARYNGODON IDELLA)**

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Cadmium is the main pollutant of industrial effluents. Discharge of industrial wastes and urban runoff in water bodies increases cadmium level in water day by day. It exerts dangerous effects on aquatic life like fish. Fish can produce antioxidants and detoxifying enzymes in response to heavy metals such as cadmium (Cd). The main objectives of the study were to evaluate the bioaccumulation of Cd in brain and muscles of fish (grass carp) and to assess the quantity of various detoxifying enzymes produced in response to accumulated quantity of Cd. Fishes were exposed to four concentration of Cd (1, 5, 10 and 15 ppm) for 14 days. Accumulation of Cd and quantity of detoxifying enzymes in brain and muscle was estimated after 1, 7 and 14 days of treatment. Accumulation of Cd in brain and muscle increased with the increase of concentration and exposure duration. Among detoxifying enzymes, activity of catalase also increased with increase of exposure time and metal concentration in brain and muscle. The quantity of glutathione S-transferase increased after 1 day exposure and decreased after 7 and 15 days of exposure. In brain, acetylcholinesterase activity decreased with time at all doses of Cd. While in muscle, increased in Acetylcholinesterase activity was noted only at 1ppm after 1, 7 and 15 days. Cytochrome P450 showed did not show any response to different concentration and exposure time. The findings of present study concludes that cadmium can accumulate in muscle and brain of *Ctenopharyngodon idella* and can induce the activation of catalase and to some extent glutathione S-transferase and the inhibition of AChE and Cytochrome P450 under the influence of metal ion concentration.

**SEASONAL COMPOSITION AND DIVERSITY OF MARINE FAUNA TRAPPED IN  
BEACH SEINE NET OPERATED AT DAM, SONMIANI BAY, BALOCHISTAN**

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The mangroves are important shelter and restrain large concentrations of finfish, shrimp, crabs water birds and other groups of marine fauna. This study aimed to evaluate the marine invertebrate and finfish fauna with the help of beach seine during experimental survey from Damb Mangrove creeks area. A total of 1843 specimens of comprising 117 finfish species, representatives of 39 families were caught by Beach seine. Family Engraulidae (25.88%), Mugillidae (13.02%) and Clupeidae (12.91%) were found the most abundant families in this study. The finfish diversity (H') was ranged from 2.6 to 4.2 and evenness (J') was ranged from 0.56 to 0.92. A total of 305 specimens of comprising 34 invertebrate species, representative of 20 families were captured. The family Portunidae (37.05%), Penaeidae (29.51%) and Matutidae (10.82%) were the most abundant invertebrates families. The diversity (H') was ranged from 1.37 to 2.83 and evenness (J') was ranged from 0.69 to 1.01. General Linear Model (GLM) analysis of variance (ANOVA) was used to

test diversity and equitability for differences between seasons, fauna and their interaction. The diversity was showed a significant differences between fauna ( $F_{1, 23} = 33.24$ ;  $P < 0.05$ ). Anthropogenic activity and jetty design also affect the occurrence of fauna and latter activity cause erosion along the coast of the Sonmiani Bay.

**ASSESSMENT OF HEAVY METAL ACCUMULATION IN *OPUSIA INDICA*  
(OCYPODOIDAE: CAMPTANDRIIDAE) COLLECTED FROM MUDFLATS OF  
PAKISTAN**

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*Opusia indica* belongs to the family Camptandriidae and common inhabitants of mangrove intertidal and mudflats areas. These are small, sluggish, burrowing and deposit feeders crab. Their bioturbation behavior plays an important role in material cycling in sediment. We hypothesized that these crabs are nourished by organic matter extracted from sediment, therefore, they may be indicated the substrate contaminations and accumulate these contaminants in their body in higher amounts. Hence, to evaluate the heavy metal concentrations in crab as reference to heavy metal contaminations in sediment *O. indica* collected from two mudflats *i.e.* Port Qasim and Sonari, Pakistan. Iron ranged from 324.3 – 494.1  $\mu\text{g g}^{-1}$ , Copper ranged from 40.9 – 98.0  $\mu\text{g g}^{-1}$ , Zinc ranged from 73.94 – 82.56  $\mu\text{g g}^{-1}$ , Cobalt ranged from 18.70–26.97  $\mu\text{g g}^{-1}$ , Chromium ranged from 7.40 – 10.94  $\mu\text{g g}^{-1}$ , Nickel ranged from 16.12 – 23.59  $\mu\text{g g}^{-1}$ , Lead ranged from 24.62 – 26.59  $\mu\text{g g}^{-1}$  and Cadmium ranged from 1.86 – 2.06  $\mu\text{g g}^{-1}$  in crab. The bioaccumulation factor (BAF) of metals in crabs was evaluated to compare the heavy metal concentrations in crab with sediments. At Port Qasim area, BAF of all metals exceeds from 1.0, except Cr and suggested that metals accumulate very vigorously in crabs as compared to Sonari. The present results indicated the ecological significance of crab, *O. indica*, as potential accumulator species for metal contamination in mudflats of Pakistan.

**AN EVALUATION ON CYPERMETHRIN-INDUCED TOXICITY UNDER  
SUPPLEMENTATION OF SELENIUM (SODIUM SELENITE) IN DRINKING WATER: A  
STUDY IN LABORATORY RATS**

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Alpha-Cypermethrin is a synthetic pyrethroid insecticide used worldwide in agriculture and as home pest control. Toxicity of these insecticides has been implicated in non-target organisms due to their widespread and unchecked use. The aim of this study was TO determine the hematological, biochemical, histopathological and other related physiological changes in female Sprague-Dawley rats following short-term (21 days) oral exposure of cypermethrin and its possible attenuation by selenium. Twenty female rats were divided randomly into four equal groups. Group 1: (1ml corn oil; vehicle); group 2: (55mg/kg b.w cypermethrin); group 3 (1ppm Sodium Selenite),

and group 4: was given (cypermethrin + sodium Selenite). Results showed that cypermethrin led to a significantly ( $p < 0.05$ ) increased activity of liver enzymes ALT and ALP. Total bilirubin, urea and creatinine were insignificantly increased. Significant ( $P < 0.05$ ) decreases occurred in RBC counts, hemoglobin concentration (Hb) and hematocrit (Hct) indicating that rats were suffering from anemia. Selenium co administration with cypermethrin alleviated the harmful effects of cypermethrin and restored the levels of above mentioned parameters comparable to those found in the control group. Histologically, cypermethrin caused degeneration of hepatic parenchyma, congestion and vacuolation of cells and dilation of sinusoids. Renal architecture demonstrated congestion in glomeruli and vacuolation in tubular epithelium. Similarly co-administration of cypermethrin with selenium restored the levels of following minerals K, Na, Mg, Cu, Zn and Se brain, heart, liver, kidney, spleen and blood tissues. The present study appears promising as regards protective effect of selenium against cypermethrin toxicity. However further studies are required to evaluate the protective effects of selenium at cellular level.

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**EFFICACY OF PHOSPHINE AGAINST *TROGODERMA GRANARIUM* OVER A RANGE OF PHOSPHINE CONCENTRATIONS AND EXPOSURE PERIODS: EFFECTS ON METABOLITES**

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The efficacy of phosphine over a range of sub lethal concentrations ( $LC_{10}$ - $LC_{40}$ ) and exposure periods (24-144 hours) at 35°C and 60±5% RH have been studied against adult beetles of a stored product pest *Trogoderma granarium*. The present study will provide the most effective combination of phosphine concentration and exposure period capable of controlling infestation of *T. granarium* (Everts). The  $LC_{50}$  values for adult beetles of Lahore and Gujranwala populations were 4.5 and 4.87 ppm, respectively. Gujranwala population showed 100% mortality at its  $LC_{30}$  (4 ppm) for the exposure periods of 120 and  $LC_{40}$  (4.5 ppm) for 96 hours, while adult beetles of Lahore population exhibited 100% mortality at its  $LC_{30}$  (3.5 ppm) for the exposure periods of 96 and  $LC_{40}$  (4 ppm) for 72 hours exposure. It was revealed from the comparisons of lethal concentrations ( $LC_{10}$ - $LC_{40}$ ) that tolerance factor in adult beetles of both populations decreased as the concentrations of phosphine increased. The results also showed that exposure period have significant interactions with phosphine toxicity irrespective of phosphine concentrations. The effect of sub lethal dose ( $LC_{20}$ ) of phosphine on the intermediary metabolites over various exposure periods (24-120 hours) has also been investigated. The glycogen, lipids, DNA and RNA contents were significantly decreased in adult beetles of both populations as exposure period was increased from 24-120 hours, while free amino acids and glucose contents were increased in Gujranwala population throughout the period. Soluble proteins in Gujranwala population, free amino acids and glucose contents in Lahore population was first increased after exposure to sub lethal dose of phosphine but then started to decrease after 72 hours exposure period. Soluble protein contents of Lahore population was first raised after exposure to sub lethal dose but then started to decrease after 48 hours exposure. These metabolic derangements induced by phosphine over various exposure periods provide a raw biochemical data to adopt better control strategy by regulating exposure period for both populations of this stored grain pest.

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### HISTOPATHOLOGICAL ASSESMENT AND HEAVY METALS CONTENT OF FISH *CHANNA PUNCTATA* COLLECTED FROM POLLUTED WATER

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Salts and heavy metals are used for leather tanning. Heavy metals are considered as the most significant and persistent pollutants in nullahs and sediments that receive tannery wastewater. The current study was conducted to access the damage caused by industrial effluents to local fish population. Bottom dweller carnivore fish *Channa punctata* were collected from nullahs around Wazirabad. Control samples from non-polluted sites were also collected for comparison. The concentration of both the macronutrients (Na, K, Mg, Ca) and heavy metals (Fe, Cr, Cu, Co, Mn, Ni, Zn, Pb, Cd) were determined through atomic absorption spectrophotometry. Gills and heart were assessed for histomorphological damage. Results were compared using the Student's t-test. Results clearly indicated greater concentrations of macro- and microelements in gills, skin, heart, brain, spleen tissues in polluted sites fish. In the gills following was the trend: Ni ( $p<0.05$ ), Cr ( $p<0.05$ ), Co ( $p<0.05$ ), Cd ( $p<0.05$ ), Na ( $p<0.05$ ), K ( $p<0.05$ ). For skin: Na ( $p<0.05$ ), Ni ( $p<0.001$ ), Cr ( $p<0.05$ ), Co ( $p<0.05$ ), Cd ( $p<0.05$ ). In the heart tissues: Ni ( $p<0.05$ ), Co ( $p<0.001$ ), Pb ( $p<0.05$ ), Zn ( $p<0.05$ ), Fe ( $p<0.05$ ), Na ( $p<0.001$ ), K ( $p<0.001$ ), Ca ( $p<0.05$ ) concentration were significantly higher. In the brain significant differences were found for: Fe ( $p<0.001$ ), Cr ( $p<0.05$ ), Co ( $p<0.05$ ), Cd ( $p<0.05$ ), K ( $p<0.05$ ). In spleen increase was found in Zn ( $p<0.05$ ), Na ( $p<0.05$ ), K ( $p<0.05$ ), Mg ( $p<0.001$ ). Histologically gills of polluted water fish showed degeneration of pillar cells and thickening of primary lamellar epithelium, lamellar disorganization, hypertrophy of the lamellar epithelium and epithelium rupture with hemorrhage. In heart atrophy, degeneration, fragmentation and hemorrhage of myocardial muscle fibers were found. The present study provided ample evidence that industrial effluents are potentially hazardous to ecosystem and can readily pass to human bodies via food chains.

### HISTOPATHOLOGICAL EFFECTS AND ELEMENTAL CONCENTRATION OF BODY TISSUES OF RANID FROG INHABITING POLLUTED WATER SITES

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Anthropomorphic activities have direct effects on amphibian population in the wild. Wildlife gets exposed to toxic metals when receptors and contaminants occur together at the same time and place. Almost 50% of total pollution is caused by surface water due to the presence of toxicants. Pollutants have direct and indirect effects on physiological functions of amphibians. The present study was carried out on Korang river Islamabad/Rawalpindi. Five samples of frogs (*Rana clamitans*) were captured from non-polluted sites of suburban ponds present at least 5 kilometers away from the polluted sites under study. Two different sites were selected for the collection of water samples along the Korang river in the month of march and October to study the toxic effects of effluents drained into Korang river in pre and post monsoon season. Seven metals including Zn, Cd, Pb, Mn, Fe, Ni, Cr were estimated in water samples. Same metals were estimated in tissue samples including heart, stomach, liver, muscle, kidney and gonads. Same tissues were processed

for histopathological studies to assess the cellular damage. Paired t-test and Pearson's correlation were carried out.  $P < 0.05$  was considered significant. In October, concentration of Zn, Pb and Cr were found significantly raised ( $P < 0.001$ ) in water samples as well as heart, stomach, liver, muscle, kidney and gonads ( $P < 0.001$ ) taken in March. Histopathologically, all tissues showed significant cellular damage; intercalated disc disrupted in the heart, mononuclear infiltration was evident in stomach tissue, sarcolemma disrupted in muscles, glomeruli found shrunken in the kidney tissue and excessive tissue congestion was found in liver tissue while abnormal follicles and cytoplasm regression were the outcome in ovary tissue. The study shows that leaching of contaminants causes negative physiological effects on the amphibians inhabiting at these study sites of Korang river.

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***PISTACIA CHINENSIS* EXTRACT PROTECTS ARSENIC INDUCED REPRODUCTIVE TOXICITY IN ADULT MALE SPRAGUE-DAWLEY RATS**

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*Pistacia chinensis* is an antioxidant plant used in the treatment of many diseases. The present study was intended to scrutinize the protective efficacy of ethanol extract of *Pistacia chinensis* against arsenic induced damage in rat testes. Forty adult male Sprague-Dawley rats were randomly distributed into four equal groups (n=10/group). The first group served as a control. The second group of rats was treated with sodium arsenite at the dose of 40 ppm in drinking water. The third group served as a positive control and received an oral dose of (300mg *Pistacia chinensis*/kg BW/day). In the fourth group, *Pistacia chinensis* extract (300 mg/kg) was co-administered with arsenic (40 ppm in drinking water). All the treatments were carried out for 30 days. Arsenic treatment resulted in a significant ( $p < 0.05$ ) decrease in plasma and intra-testicular testosterone concentrations. Arsenic treatment induced significant ( $p < 0.05$ ) escalation in the thiobarbituric acid reactive substance (TBARS), and marked reduction in protein concentrations, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GSR) activities in testicular tissue of rat. Moreover arsenic treatment significantly ( $p < 0.05$ ) induced sperm DNA damage. However, *Pistacia chinensis* treatment significantly ( $p < 0.05$ ) recovered all the damages caused by arsenic. On the basis of these findings, it was concluded that *Pistacia chinensis* extract may be used to ameliorate arsenic induced reproductive toxicity.

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**EFFECT OF *CARISSA OPACA* FRUIT EXTRACT ON LEAD INDUCED TESTICULAR TOXICITY IN MALE RATS**

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Lead poisoning has been recognized as a major public health risk particularly in developing countries. So the principal aim of this study was to evaluate the protective effect of *Carissa opaca* fruit extract on lead induced testicular toxicity in male rats. Forty adult male Sprague-Dawley rats

were randomly distributed into four equal groups (n=10/group). The first group served as a control. The second group of rats was treated with lead acetate at the dose of 30 ppm in drinking water. The third group served as a positive control and received an oral dose of *Carissa opaca* fruit extract (200 mg/kg). In the fourth group, *Carissa opaca* fruit extract (200 mg/kg) was co-administered with lead acetate (30 ppm in drinking water). All the treatments were carried out for 30 days. Lead treatment significantly increased testicular thiobarbituric acid reactive substance (TBARS) levels while catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and glutathione reductase (GSR) activities, and plasma and intra-testicular testosterone concentrations, were decreased significantly. Lipid peroxidation (LPO) was significantly suppressed and depleted antioxidant defense mechanism was restored by the *Carissa opaca* fruit extract treatment. Also *Carissa opaca* fruit extract treatment resulted in a marked increase in plasma and testicular testosterone concentrations. On the basis of these findings, it was concluded that *Carissa opaca* fruit extract may be used to ameliorate lead induced reproductive toxicity.

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**POSSIBLE ROLE OF ESTERASES IN DEVELOPMENT OF TOLERANCE AGAINST  
PHOSPHINE IN PHOSPHINE EXPOSED POPULATIONS OF *TROGODERMA*  
*GRANARIUM* FROM PUNJAB**

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Development of resistance to pesticide has become a universal phenomenon in all stored product pests which hampers the pest control program. It is very important to know the mechanism of resistance in insects to launch a successful pest control strategy. The present study was aimed to determine the possible role of esterases in development of tolerance/resistance in phosphine-tolerant populations of wheat grain pest, *Trogoderma granarium* collected from various storage facilities of Punjab. The level of various esterases (Total esterases, Carboxyl esterases, Choline esterases, Acetylcholine esterases and Aryl esterases) in 4<sup>th</sup>, 6<sup>th</sup> instar larvae and adult beetles of phosphine-tolerant populations viz., Mandi Bahauddin-I (MBDIN-I), Mandi Bahauddin-II (MBDIN-II), Gujrat, Gujranwala and Sargodha of *T. granarium* was evaluated by using susceptible population as a control. The level of TE, CE, ChE, AChE and AE activities were significantly increased in all tolerant populations with reference to susceptible population. The TE and CE activities in 4<sup>th</sup>, 6<sup>th</sup> instar larvae and adult beetles were significantly different from each other in all phosphine-tolerant populations. In 4<sup>th</sup> instar larvae the ChE activity of various tolerant populations was significantly different from each other except (Sargodha and MBDIN-I population) and (MBDIN-II and Gujrat population), likewise in 6<sup>th</sup> instar larvae the ChE activity was significantly different from each other except Gujrat population which was not significantly different from MBDIN-II and Sargodha populations at P<0.05. In adult beetles MBDIN-I was not significantly different from Gujrat and Sargodha populations while rest of tolerant populations were significantly different from each other. In 4<sup>th</sup> instar larvae, the AChE activity was significantly different from each other except (Sargodha and MBDIN-I population), likewise in 6<sup>th</sup> instar larvae, AChE activity was significantly different from each other in various populations except (Sargodha and Gujranwal population). In adult beetles non significant difference was observed in AChE activity of MBDIN-II and Gujrat population likewise Sargodha population was not significantly different from Gujrat and Gujranwala population. The AE activity was significantly different in 4<sup>th</sup> instar larvae of all tolerant populations likewise the 6<sup>th</sup> instar larvae of all tolerant populations also

possessed significantly different AE activity. In case of adult beetles, MBDIN-II and MBDIN-I had non-significant difference at  $P < 0.05$ . The increased level of esterases in phosphine tolerant populations as compared to susceptible population has pointed some correlation between esterase and phosphine tolerance. Further studies are needed to investigate the formation of other phosphine derivatives in insect body (if any) which may be responsible for increase in esterase levels in phosphine-tolerant insects.

**BIOSIMILARITY OR BIOEQUIVALENCE STUDY OF LOCAL BRAND FILGEN 30MIU  
(FILGRASTIM GCSF) AGAINST REFERENCE STANDARD BRAND LEADER  
NEUPOGEN 30MIU (FILGRASTIM)**

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Filgrastim is a human granulocyte colony stimulating factor (G-CSF) produced by recombinant DNA technology. G-CSF regulates the production of neutrophils within the bone marrow; endogenous G-CSF is a glycoprotein produced by monocytes, fibroblasts, and endothelial cells. G-CSF is a colony stimulating factor which has been shown to have minimal direct in vivo or in vitro effects on the production of other haematopoietic cell types. NEUPOGEN (filgrastim) is the name for recombinant methionyl human granulocyte colony stimulating factor (r-metHuG-CSF). For bioequivalence study five samples of each local brand of Filgen 30MIU and brand leader of Neupogen 30MIU were taken and analyzed against different parameters such as physical appearance, pH, SDS-PAGE, Western blot, HPLC and Biological activity assay. Locally produced brand of Filgen 30MIU when analyzed and compared physiochemically with the brand leader of Neupogen 30MIU and statistical analysis was rendered which showed both products biosimilar and bioequivalent. It was concluded that biosimilar product Local brand of Filgen 30MIU is chemically and biologically similar to the reference product Brand Leader of Neupogen 30MIU. For biological response 'In vitro' study of both products Local brand of Filgen 30MIU and Brand Leader of Neupogen 30MIU was performed on micro plate reader and it produced biosimilar results

**INCLUSION OF DIFFERENT LEVELS OF AFLATOXIN B<sub>1</sub> IN FEED AND ITS EFFECTS  
ON GROWTH, SURVIVAL AND HISTOLOGY OF FINGERLING CATLA CATLA**

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The experiment was conducted on *Catla catla* weighing average  $38.31 \pm 3.0$ g each for the period of 90 days. 120 *Catla catla* fingerlings were randomly stocked into twelve circular tanks (10 fish per tank) containing 240 litre of water. All trial contained 5 treatment/test groups and control with two replicates. Fungus was grown on rice media. On maturity of culture fungus, with the presence of Aflatoxin and mixed in prepared diet at different concentrations to elucidate its toxicity



on growth performance of *Catla catla*. Prepared diet was 25% crude protein which was divided into five equal parts. Part I was reserved for control group and remaining five groups received Aflatoxin in different concentrations. These diets were orally offered to fingerling *Catla catla* to test their effect on growth, general health and toxicity on vital organs of experimental fish. Samples from each group were dissected and the kidneys, liver and intestine were taken for histopathology. The results showed that *Catla catla* in control group grew significantly higher than all its counterparts (T<sub>2</sub>, T<sub>4</sub>, T<sub>1</sub>, T<sub>3</sub> and T<sub>5</sub>). The survival was 100 % in all the groups except T<sub>4</sub> and T<sub>5</sub> where mortality started with maximum (30%) in T<sub>5</sub>. Significant reduction in weight gain in 40 and 50 ppb concentrations of Aflatoxin B<sub>1</sub> when compared with control. Poor growth and drastic histopathological changes in *Catla catla* were found. DO rapidly declined when fish was exposed to Aflatoxin even to very minute concentrations. The severity of hepatic lesions in 40 and 50 ppb concentrations finally suppressed appetite and food digestion after two months of administration which could have affected weight gain, FCR and SGR. The growth increments were compared among different treatments, differences remained insignificant from T<sub>2</sub> to T<sub>5</sub>. However, the fish in control and T<sub>1</sub> grew equally and significantly higher than all the other treatments. The control group remained at the top while fish in T<sub>5</sub> grew the least. It is concluded that 40 and 50 ppb Aflatoxin, there was prominent hepatocyte swelling with pyknotic nuclei. Liver was the principal target organ for aflatoxins. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and leads to necrosis or liver cell death.

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**ASSESSING THE LEVEL OF METAL BIOACCUMULATION IN HEPATIC AND RENAL TISSUES OF FRESHWATER FISH, *CYPRINUS CARPIO* COLLECTED FROM RIVER CHENAB BY USING OXIDATIVE STRESS BIOMARKER CATALASE**

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Water contamination is a severe environmental issue and has worse effects on fish health. The proposed study was designed with an objective to measure the level of various metal (*i.e.* Pb, Cd, Ni, Cr, Zn, Co and Cu) accumulation in riverine *Cyprinus carpio* hepatic and renal tissues by measuring the activity of catalase enzyme which is an important part of antioxidant system and considered an excellent biomarker for indication of water contamination. For this purpose proposed fish samples were collected from River Chenab important site, Trimmu Headworks. For the comparison of the inferences, the proposed fish samples were also collected from control conditions *i.e.* pond raised fish. The activity of extracted and homogenized hepatic and renal tissues catalase enzyme were determined by measuring its ability to decompose H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> molecule at 240nm with the help of spectrophotometer. The inferences of the present study revealed higher hepatic (134.3±0.305 U mL<sup>-1</sup>) and renal (121.07±1.37 U mL<sup>-1</sup>) catalase enzyme activity in pond fish compared to the riverine fish liver (107.2±0.435 U mL<sup>-1</sup>) and kidney (104.4±0.41 U mL<sup>-1</sup>) catalase enzyme activity. The statistical analyses showed significant difference (p≤ 0.05) between pond raised and riverine captured *C. carpio* hepatic and renal catalase enzyme activity. Accumulation level of various selected heavy metals in studied body organs of *C. carpio* were observed higher in riverine fish compared to pond raised fish that indicate metal

contamination in River Chenab. The order of metal deposition in hepatic tissues was recorded as; Zn > Co > Cu > Cr > Pb > Ni > Cd in riverine *C. carpio* and Cu>Co>Zn>Ni>Cr>Pb>Cd in pond raised *C. carpio* while, deposition rate in renal tissues was recorded as; Co>Zn>Cu>Ni>Pb>Cd>Cr in riverine *C. carpio* while, Zn>Ni>Cu>Cr>Co>Cd>Pb in pond raised *C. carpio*. On the basis of present study inferences it is concluded that fish tissues and biomarkers serves as an indicator of metal contamination in aquatic environment.

#### ANTIBACTERIAL EFFECT OF ETHANOLIC EXTRACTS OF PLANTS, SPICES AND HERBS ON BACTERIAL ISOLATES FROM CONTACT LENS

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With the emergence of antibiotic era hardly five decades ago people are afflicted with global problem of increasing resistance in almost all pathogens. Medicinal plants have been used since antiquity as a primary health care system. Due to the increasing infections and elevated rates of synthetic drugs along with their side effects medicinal plants are being used by 80% of population to treat various ailments. So the present study was designed to evaluate antibacterial effect of ethanolic extracts of plants, spices and herbs against the bacteria isolated from contact lens that cause severe infections to human eye. Antibacterial activity of thirty samples of ethanolic extracts of plants, herbs and spices was tested against both gram positive and gram negative bacteria isolated from 60 samples of contact lens that were taken from different sources by disc diffusion method with the measurement of diameter of zone of inhibition around each extract. The results showed the presence of antibacterial activity of almost all the plant, herbs and spices extract with a highest zone of inhibition of 40mm against *S. aureus* by *Bacopa monnieri* and *Emblica officinalis* plant extracts while *Achillea millefolium* extract was resistant against bacteria. Among spices extract turmeric extract showed largest inhibition zone of 35mm against *Micrococcus luteus* and most resistivity was also shown by turmeric extract against *Bacillus sp.*, *P. aeruginosa* and *E. coli*. Herbs extracts showed a maximum zone of inhibition of 25mm against *K. pneumoniae* with mint extract whereas the most sensitivity was observed against *M. luteus*. All the samples exhibited antibacterial activity against *S. aureus*, *M. luteus*, *Bacillus sp.*, *P. aeruginosa*, *Klebsiella pneumoniae* and *E. coli* except *Achillea millefolium* plant that showed resistivity against all bacterial isolates.

#### CHARACTERIZATION OF ANTIBIOTIC RESISTANT GENE IN STAPHYLOCOCCUS AUREUS ISOLATED FROM SURGICAL WOUNDS

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The present study was carried out to determine the prevalence of different pathogens in 300 surgical wounds, and their antimicrobial susceptibility patterns. Pus swab from each patient was collected aseptically, and inoculated on culture media. Isolates were characterized and identified.

Antibiotic susceptibility patterns were determined using the Kirby-Bauer diffusion method. Out of a total of 300 surgical wound specimens analyzed, 208 samples were positive culture and rank of the organisms was as follows: *Staphylococcus aureus* were the most frequent pathogen with 103 isolates (49.51%), followed by 33 isolates (15.86%) of *pseudomonas aeruginosa*, 27 isolates (12.98%) of *klebsiella*, 25 isolates (12.01%) of *proteus* and 20 isolates (9.61%) of *E.coli*. The antibiotic susceptibility of *S. aureus* were carried out and showed that 103 isolates of *S. aureus* were resistant to Augmentin (by 65.04%), Sparfloxacin (59.22%), Amikacin (57.28%), Cefuroxime (57.28%), Cefotaxime (56.31%), Ceftazidime (53.39%), Fusidic acid (48.54%), Ciprofloxacin (39.80%), Methicillin (37.86%), Gentamicin (28.15%), Meropenem (13.59%), and Imipenem (5.82%). PCR assay for the detection of clinically relevant antibiotic resistance gene of *S. aureus* was done. Conditions were optimized to amplify fragments of *mecA* (encoding methicillin resistance) gene in PCR amplification and its sequencing was done commercially which showed insertion at three sites *i.e.* 480-481: T, 484-485: T, 464-465: G. PCR assay offered a rapid, simple, and accurate identification of antibiotic resistance profiles and could be used in clinical diagnosis as well as for the surveillance of the spread of antibiotic resistance determinants in epidemiological studies.

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#### IN HOUSE METHOD OPTIMIZATION AND VALIDATION OF PARTICLE INDUCED X-RAY EMISSION (PIXE) FOR HEAVY METALS DETECTION FROM HUMAN HAIR

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The objective of this study is to Optimize and Validate to determine the Aluminum (Al), Calcium (Ca), Potassium (K) and Sulfur (S) metals concentrations in the human hair of different industries workers like Pharmaceutical industry, Textile Industry and Paint Industry, the data has been presented into the three groups. Methods were developed to detect heavy metals from Human hair. In the group 1 from the pharmaceutical industry the mean for Aluminum (Al) metals was 575.1 and show significant differences the P – value for Aluminum was  $p < 0.05$ , the mean for Calcium (Ca) was 9.1 were non-significant different in this group the P – value was  $p > 0.05$ , the mean for Potassium (Ca) metal was 11.1 and show non-significant differences the p – value was  $p > 0.05$ , the mean for Sulfur (S) metal was 190.7 show significant differences and p – value was  $p < 0.01$ . In group data 2 from textile industry the mean for Aluminum (Al) metal was 116.9 show non-significant differences the p – value was  $p > 0.05$ , the mean for Calcium (Ca) was 1.7 and show the significant differences the p – value was  $p < 0.01$ , the mean for Potassium (K) was 3.2 and found non-significant differences and the p – value was  $p > 0.05$ , the mean for Sulfur (S) 17.7 show non-significant differences the p – value was  $p > 0.05$ . In group data 3 from paint industry the mean for Aluminum (Al) was 103.7 which was non-significant the p – value was  $p > 0.05$ , the mean for Calcium (Ca) was 1.7 which having significant differences the p – value was  $p < 0.01$ , the mean for the Potassium (K) metal was 2.3 and was non-significant different the p – value was  $p > 0.05$ , the arithmetic mean for Sulfur (S) metal was 8.87 and was non-significant different the p – value was  $p > 0.05$ . It is concluded that the concentration of Aluminum (Al) metals was high in all three industries which determined that the Aluminum (Al) metal having much more exposure to industrial workers.

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**FACTORS AFFECTING FIBER DEVELOPMENT IN COTTON**

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Cotton fiber and seed hair are single celled trichomes that originate from outer integument of ovule, undergo rapid and synchronous elongation during fiber development. Fiber development occurs in four distinct and overlapping stages; initiation, elongation (primary cell wall formation), secondary cell wall formation and maturation. Genes involved in fiber elongation are *CEL*, *CelA1*, *CelA1*, *Exp1*, *ACT 1*, *BG*, *Pel*, *SuS1*, *LPT3*, *GhE6*, *pGhEX1*, *GhCESA1* whereas transcription factors like MYB, WRKY, AP2/EREBP, *C<sub>2</sub>H<sub>2</sub>* and bHLH families may have vital role in fiber cell initiation. The hormones in plant *i.e.*, cytokinin, abscisic acid, ethylene, brassinosteroids (BR), auxin and gibberellins also have their impact on fiber development and initiation whereas temperature is critically important for cotton boll maturation. Optimum temperature for fibre elongation is slightly lower than optimum temperature for boll maturation. Carbohydrate like malate and soluble sugars are responsible for increasing turgor, whereas expansin and endoglucan transferase (EXGT) are involved in cell wall extension. Transformation of fibre related genes in cotton from other plants having better fibre quality traits may also help to improve the fibre elongation.

**PREVALENCE, RISK FACTORS AND ANTIMICROBIAL RESISTANCE OF E.COLI ISOLATED FROM UTI IN PREGNANT FEMALES**

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The present study deals with isolation of *E. coli* causing UTI among pregnant women, its antibiotic resistance pattern and to find the association between the risk factors contributing towards occurrence of infections. The study was also conducted to compare the incidence of UTI during different trimesters of pregnancy. A total of 300 urine samples were collected from pregnant ladies visiting for their antenatal checkup in different hospitals of Lahore. Details of patients regarding age, socioeconomic status, personal hygiene, previous history of UTI, trimester of pregnancy, habit of retaining urine, history of diabetes, history of catheterization, history of symptoms of UTI and frequency of sexual activity were noted. Bacteria were isolated and biochemically identified. Antibiotic sensitivity pattern of *E. coli* was tested by using Kirby Bauer disc diffusion method. Out of 300 urine samples, 117 showed positive growths for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas spp.* *E.coli* was the most common pathogen and the prevalence of UTI was 23 %. *E. coli* showed 73.91 % resistance to doxycycline and maximum sensitivity to imipenem 84.05 %. Molecular characterization by using 16S rRNA gene sequencing technique was performed for further confirmation of strain. It exhibited 93 % similarity with *E. coli* 042. The selected strain was confirmed as *E. coli*. It was observed that pregnant females were more prone to UTI in 2<sup>nd</sup> trimester followed by 1<sup>st</sup> and 3<sup>rd</sup> trimester of pregnancy.

### ROLE OF MICROBIAL FORENSICS IN PAKISTAN: BACTERIAL BLIGHT, A TEST CASE

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Awareness for a crop bioterror incident begins with start of the threat symptoms, which must lead to the progress of a plan to thwart. However, most of Pakistani scientists and crop producers have not focused on the option of deliberative plant pathogen introduction but instead they use their expertise for the prevention of natural introductions and the improvement of cost-effective strategies for disease management. It's also interesting that on the name of genetically modified feeds, high yield varieties and hybrids; we are also importing the bio terrorism. Are we going on right path? In present study, we propose the integration of the traditional knowledge of plant pathology and the advanced field of forensic science for envisioning the birth of the new field; microbial forensics. The possible threats to plant resources and development of national security plan for microbial forensics that addresses crop-microbial bioterrorism. Here we are presenting a model research related to forensics analysis of a crop disease "bacterial blight" caused by *Xanthomonas oryzae*, in rice which is the second most important crop of Pakistan. By commencing this work, we exercised all the steps required for microbial forensics and we have also looked on pros and cones of future of microbial forensics studies in Pakistan.

### ISOLATION AND ESTIMATION OF LYMPHATIC PROTEINS PROFILE OF TILAPIA REARED IN POND WATER

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The study was conducted for the qualitative and quantitative comparison of lymphatic proteins of the tilapia reared in freshwater pond. The lymphatic system plays a pivotal role in the development of the adaptive immune system, an important circulation of nutrients, antibodies and electrolytes etc. The development of functional T and B –cells from the thymocytes is a crucial step in development of functional immune system and the organs like spleen, kidney and thymus are vital sites to study the lymphatic proteins. Different lymphatic proteins isolated from teleosts but still a little bit known about these proteins. Estimation of proteins from tilapia was done first time in Pakistan. Isolation of lymphatic proteins was performed after tissue rupturing and all the cellular debris was removed. The determined average comparative quantitative estimation of lymphatic proteins concentrations in six months tenure in male tilapia's organs was as Testes (8.705 mg/ml) > Kidney (7.577 mg/ml) > Gills (7.556 mg/ml) > Spleen (7.455 mg/ml) > Alimentary Canal (7.243

mg/ml) and in female tilapia as Ovaries (9.098 mg/ml) > Kidney (7.742 mg/ml) > Spleen (7.548 mg/ml) > Gills (7.532 mg/ml) > Alimentary Canal (6.920 mg/ml). High concentration of protein was investigated in reproductive organs (testes, 9.41 mg/ml and ovaries, 9.82 mg/ml) in both sexes. SDS- and native-PAGE bands profile of isolated proteins was indicated the range of bands from 10 kDa – 170 kDa with varying intensity of total 30 – 40 bands. The comparative analysis reducing and non-reducing PAGE result the remarkable differences between different bands.

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#### TARGETED GENE SILENCING VIA COMPUTATIONALLY ACTIVE MICRO RNAs AGAINST POTATO VIRUS Y (PVY)

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*Potato virus Y* has emerged as a threatening problem in all potato producing areas around the globe. It produces a wide range of disease symptoms in various potato cultivars leading to yield and quality reduction. During last 30 years, signification changes in PVY strain has been observed with an increased incidence associated with potato tuber damage. Present study focused on the application of computational approaches to find out Potato miRNAs targets in PVY genome. Total 343 mature miRNAs were retrieved from miRbase database and searched for their target sequences in PVY essential genes using multiple approaches. Effective Potato miRNAs against viral mRNA targets have an antiviral activity which was observed on the basis of complementarity of miRNA-mRNA, leading to either translational inhibition or mRNA cleavage or both. About 86 miRNAs were found targeting PVY genome at 151 different positions. Moreover, only 36 miRNA potentially targeted the PVY genome at 101 loci. CI gene of PVY genome was targeted by 32 miRNAs followed by NIb, HC-Pro, NIa-Pro, Vpg and CP by 26, 19, 18, 16 and 13 miRNAs respectively. Most importantly, we found 5 miRNAs (miR160a-5p, miR7997b, miR166c-3p, miR399h and miR5303d) could target CI, NIa, NIb-Pro, HC-Pro, CP and VPg genes of PVY. These 5 miRNAs have effective resistance against PVY infection in genetically engineered cotton.

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#### CONVENTIONAL AND MOLECULAR DETECTION OF PLASMODIUM SPP. IN HUMANS

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Conventional and molecular detection of plasmodium, causative agent of malaria was carried out, aiming at comparing the sensitivity of both the protocols. For conventional diagnosis, thin blood smear from 50 malaria suspected victims from Umerkot, Tandojam and adjoining areas were prepared and stained with Romanovsky stain, whereas molecular confirmation on same sample was done by Polymerase Chain Reaction (PCR). For identification of species, restriction enzyme (AluI) analysis of the amplified product was performed. Blood smear examination indicated 18 (36%) cases were positive with Plasmodium parasite, whereas PCR analysis detected plasmodium at 850 base pairs (bps) showing that only 6 (12%) blood samples infected with plasmodium. When

PCR positive samples were subjected to AluI enzyme, the protocols revealed that all victims were positive with *Plasmodium falciparum*. Comparison of conventional and molecular techniques revealed that 12 (66.60%) false Positive cases were detected by microscopy, whereas PCR assay did not conformed to these results. Statistical analysis on the prevalence of malaria revealed non significant difference from Umerkot and Tandojam at  $P > 0.05$  ( $2 = 1.815$ ;  $P = 0.1779$ ) with 1 degree of freedom (df) whereas PCR results demonstrated significant difference at  $p < 0.05$  ( $2 = 4.649$ ;  $P = 0.0303$ ) with 1 df.

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#### **EFFECTS OF ALOE VERA EXTRACT ON THE HAIR GROWTH OF EXPERIMENTAL MICE**

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*Aloe vera* is known for its medicinal benefits and is widely used plant in some remedies even in hair growth. With view to test the village wisdom, extract of aloe Vera was applied on the shaved abdomen of 3 experimental mice and one was left with no Aloe Vera treatment for 15 days. The results indicated that the experimental mice that received topical application with Aloe Vera showed fast growth of hair as compared to un-treated the control.

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#### **STUDY ON OCCURRENCE OF BABESIOSIS IN BUFFALOES AT HYDERABAD CATTLE COLONY: A MOLECULAR DIAGNOSTIC APPROACH**

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Due to the multipurpose qualities, buffaloes are rightly called as "Black Gold of Pakistan" and are exposed to various threats; ecto-parasites may be ranked at top. Study mainly was focused on sensitivity comparison between thin blood smear diagnostic techniques with that of Polymerase Chain Reaction (PCR). Out of 100 randomly selected buffalo from five different farms, 20 were found with babesia infection in their blood with 20% prevalence percentage by conventional method (smear method) and 17% prevalence by PCR technique. Out of 100 buffalo examined, 18 were tick-infested, among which the prevalence percentage found by smear method as 100% and in 82 tick-free buffalo this prevalence was recorded as 2.5% only while by PCR technique this prevalence was recorded as 2% and 15% in tick-free and tick-infested buffalo respectively. It was concluded from the study that the babesiosis infection is present in buffaloes at Hyderabad Cattle Colony and is higher in tick-infested buffaloes than tick-free buffaloes. Besides this, tick-infested buffaloes are 1.5 times more at risk to babesia infection as compared to tick-free buffaloes. Moreover, it was revealed that PCR assay was more sensitive diagnostic approach for Babesia species than conventional slide microscopy. Since conventional blood smear test does not help in differentiating between fact and artifact, hence, molecular surveillance may be given due consideration.

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**CHICKPEA FEEDING INTAKES PROMOTES WEIGHT GAIN IN GUINEA PIGS**

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The present study was conducted to determine the effect of chickpea and carrot feeding on weight gain of guinea pigs. For this purpose, 08 guinea pigs were distributed in 02 groups (A and B) with 02 treatments chickpea and carrot feeding. The observation was recorded on body weight from day 4 to day 14 (10 days) first 03 days animals were left for acclimatization. The results indicates that average body weight of guinea pigs in group A for male and female was  $448.25 \pm 0.62$  g and  $348.75 \pm 0.47$  g. The body weight gain of guinea pigs (male) in group A from day 3, 4, 5, 6, 7, 8, 9, 10 and 11 for male was  $451.25 \pm 3.72$ ,  $458.25 \pm 3.72$ ,  $465.25 \pm 3.72$ ,  $472.00 \pm 3.69$ ,  $480.50 \pm 4.55$ ,  $486.75 \pm 4.44$ ,  $495.25 \pm 4.98$ ,  $501.50 \pm 4.64$ ,  $509.25 \pm 4.71$  and  $520.00 \pm 4.56$  g, respectively. Similarly body weight of guinea pigs (female) in group A was  $350.75 \pm 3.25$ ,  $355.25 \pm 4.62$ ,  $362.25 \pm 4.62$ ,  $368.50 \pm 4.66$ ,  $375.75 \pm 4.58$ ,  $381.75 \pm 5.13$ ,  $390.50 \pm 5.32$ ,  $398.25 \pm 4.93$ ,  $406.75 \pm 4.71$  and  $417.50 \pm 5.20$  g. In case of group B (male) the average body weight gain was  $263.25 \pm 4.11$ ,  $262.75 \pm 4.28$ ,  $261.25 \pm 4.34$ ,  $261.50 \pm 4.83$ ,  $259.75 \pm 5.58$ ,  $264.25 \pm 4.36$ ,  $261.75 \pm 3.68$ ,  $260.50 \pm 3.68$ ,  $260.00 \pm 3.69$  and  $259.00 \pm 3.69$  g from day 1 to 10. Meanwhile, the average body weight gain of guinea pigs in group B (female) was  $335.25 \pm 3.42$ ,  $335.00 \pm 3.71$ ,  $335.50 \pm 3.84$ ,  $333.75 \pm 4.09$ ,  $333.75 \pm 3.77$ ,  $336.50 \pm 3.52$ ,  $333.75 \pm 3.77$ ,  $258.50 \pm 23.67$ ,  $256.75 \pm 23.87$  and  $255.50 \pm 23.97$  g. The data reveal that average body weight gain of guinea pigs in group A (where the animals were fed on chickpeas) were linearly increased from day 1 to day 10. While, in group B (where the animals were fed on carrots) the average body weight gain of guinea pigs was decreased during the entire period of ten days. The results further concluded that male animals gain higher body weight then the female in group A. In group B (female) achieved highest body weight gain as compared to male. The ANOVA demonstrated that significant ( $P < 0.05$ ) difference was observed in average body weight gain of guinea pigs from day 4 to day 14 among the groups A and B. No significant ( $P > 0.05$ ) difference in average body weight gain (g) of guinea pigs was noted between male and female in group A from day 1 to day 10 and in group B on day 1, 8, 9 and 10. Statistical analysis of variance further stated that average body weight (g) in group A (male and female) were significant and linear from day 4 to day 14. In group B significant difference in average body weight gain were determined between day 1 and 2 in female; while in male significant difference in average body weight gain was examined on day 1 and 9.

**TESTING EFFICACY OF ONION BULB EXTRACT AS REPELLENT AGAINST *MUSCA DOMESTICA***

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Present study was conducted to determine the efficacy of onion bulb extract as repellent against *Musca domestica* in district Hyderabad. House flies were collected from hotels/homes and identified using microscope. Identified flies were raised on a carbohydrate solution in glass jars. The selection of onion was obtained by chopping the onions in a pestle and mortar, then dribbled with the aid of simple fabric and finally centrifuged for purification. Three netted jars were used in the experimentation. The repellency of onion extract at various concentrations revealed that the



maximum repellency (100%) may be obtained at a concentration of 80%, whereas sugar solution and tap water did not show any repellent. It was observed that with an increase in concentration, the repellency also increased. Effects on human response against the smell of onion extract revealed that the odor of onion was not discovered by humans.

#### **PERVASIVENESS OF TICK-BORNE DISEASE, BABESIOSIS IN QUETTA CITY OF PROVINCE BALOCHISTAN, PAKISTAN.**

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Babesiosis is an economically important and chief arthropod-borne disease, which is commonly known as cattle fever or tick fever abundantly found throughout the world. In the present study, a total of 110 cattle's includes 82 females (cows) and 28 males (bulls) were analyzed from different farm houses of Quetta city to check the infestation of ticks as well as suffer from tick born disease that is babesiosis. According to the obtained results of the present study, cows were found to be more susceptible to severe babesiosis because out of their total observed samples (n=82), 54.8% were found to be infested. Furthermore, among these infested cows, 31.1% were suffering from severe babesiosis, while 42.2% were suffered from mild babesiosis. While in contrast, out of 28 observed bull samples, 42.8% were found to be infested. Out of these infested bulls, 33.3% were suffered from severe babesiosis, while the remaining 33.3% were suffering from mild babesiosis.

#### **EFFECT OF VITAMIN D AND VITAMIN PREMIX SUPPLEMENTATIONS ON GROWTH OF *LABEO ROHITA* AND *CIRRHINUS MRIGALA*.**

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Studies were conducted to determine the effect of vitamin D and vitamin premix on growth, survival, body composition and enzyme activity in digestive tract of yearling *Channa marulius* and *Labeo rohita*. There was single treatment and a control having two replicate ponds in each. Each pond has 250 fish. Treatment 1 was supplemented with vitamin premix @ 2% of fish feed and treatment 2 with vitamin D @ 3% of the feed. All the treatments including control had two replicate tanks. Ponds in control group were regularly fertilized at every 4<sup>th</sup> day with daily addition of artificial feed. Sampling was done after every fifteen days for weight and length measurements. Water quality parameters such as DO, salinity, electrical conductivity, pH, temperature, nitrates, phosphates and plankton productivity were regularly monitored. *Cirrhinus mrigala* in treatment group gained significantly higher weight gain ( $5.83 \pm 0.67^a$ ) than control group which weight ( $2.92 \pm 0.03^b$ ). There were significant variations in body composition too between two groups. *Labeo*

*rohita* in control group gained significantly higher weight ( $32.12 \pm 0.87^a$ ) than treatment group ( $20.76 \pm 1.03^b$ ). Crude protein was higher, fat and ash were lower in control group when compared with that of treatment group. In addition to these parameters moisture was higher ( $82.00 \pm 3.00^a$ ) in treatment group than control group ( $77.66 \pm 2.51^a$ ). Control group has higher amount of copepods, ostracodes, cyclops and other phytoplankton than treatment group.

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**EFFECT OF ARTIFICIAL FEED AND SUNFLOWER BASED FEED ON GROWTH, SURVIVAL AND BODY COMPOSITION OF *LABEO ROHITA* (ROHU) FED ON HIGH PLANT PROTEIN DIET**

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Studies were conducted to determine the effect of locally developed artificial feed on growth, survival, body composition and enzyme activity in digestive tract of *Labeo rohita*. There was single treatment and a control having two replicate ponds in each. Each pond has 150 fish. Fishes in treatment group received artificial feed containing 30% protein which was offered @ 2% body weight. Ponds in control group were regularly fertilized at every 4<sup>th</sup> day with daily addition of sunflower based feed. Sampling was done after every fifteen days for weight and length measurements. Water quality parameters such as DO, salinity, electrical conductivity, pH, temperature, nitrates, phosphates and plankton productivity were regularly monitored. Control group has higher amount of copepods, ostracodes, cyclops and other phytoplankton than treatment group. There were significant variations in body composition too between two groups. Crude protein was higher, fat and ash were lower in control group ( $59.00 \pm 3.00^a$ ,  $9.00 \pm 2.00^a$ ,  $6.33 \pm 2.08^a$ ) when compared with that of treatment group ( $53.33 \pm 3.05^a$ ,  $7.33 \pm 2.08^a$ ,  $5.33 \pm 1.52^a$ ). In addition to these parameters moisture was higher ( $82.00 \pm 3.00^a$ ) in treatment group than control group ( $77.66 \pm 2.51^a$ ). Control group displayed higher weight ( $20 \pm 11.25^a$ ) and length ( $0.6 \pm 0.40^a$ ) while treatment group displayed weight ( $13.6 \pm 7.32^b$ ) and length ( $0.5 \pm 0.44^b$ ).

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**STUDYING THE EFFECT OF INCLUSION BODIES ON THE VIABILITY OF *E. COLI***

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Over-expression of recombinant proteins in *E.coli* is widely shown to lead to intracellular accumulation of partially folded proteins into aggregates called inclusion bodies (IBs). There is this unsettled debate regarding the role of inclusion bodies whether they are toxic to the cells or they are formed in response as a coping mechanism for toxic monomers. This project studies the relationship between cell viability and inclusion bodies formed from the overexpression of Alcohol Dehydrogenase in *E. coli*. The change, in classical view of the inclusion bodies that they always have to contain unstructured proteins, to the fact that IBs may contain structured and folded proteins retaining their functionality, have made IBs relevant to Biotechnology. This has led to the change in landscape from where IBs which was always considered to be a nuisance and a thing to get rid of in Biochemistry texts to an entity which is already folded and immobilized into a form that can be readily purified and used in fermentation systems. Inclusion bodies also provide a

convenient and effective way for isolation of a protein of interest as an insoluble fraction, thus reducing the number of purification steps to recover functional proteins. Thus recombinant proteins, expressed as inclusion bodies in *E.coli* are finding application for the commercial production of proteins. In order to check the effect of inclusion bodies on cell viability pET21a (+) containing adhB gene from DH5 $\alpha$  was isolated and transformed into expression strain BL21-codonPlus (DE3) and over-expressed the adhB gene under T7 promoter of pET21a (+) by using isopropyl  $\beta$ -D-thiogalactoside (IPTG) as an inducer. After plating, Miles and Misra method was used to calculate CFUs at different time points comparing the viability between induced and uninduced cells.

#### **GENOMIC CHARACTERIZATION OF INTERFERON ALPHA GENE OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I IN ENDANGERED PUNJAB URIAL**

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The objective of this study is to characterize endangered Punjab Urial via interferon alpha-A gene. Urial samples were collected for DNA extraction from various home tracks in the Punjab province. Specific primers were applied for PCR amplification of IFN $\alpha$ -A (401bp) followed by sequencing using genetic analyzer 3130xl. Sequences were aligned and identification of single nucleotide polymorphism and phylogenetic analysis were done through BioEdit 7.0.9.0 and MEGA 6.1 software respectively. This gene was found highly polymorphic. The phylogenetic trees were constructed with other mammalian species to have insight about biological classification of the Punjab urial. The highly polymorphic nature of this gene fragment makes it potential candidate for finding molecular markers related to breed adaptation and immunity to geographic regions with various climatic conditions and pathogen load. These valuable findings on interferon alpha-A gene provided foundation for further studies on this valued and unique wild genetic resource of Pakistan.

#### **ASSESSMENT OF MICROBIAL QUALITY OF DRINKING WATER FROM KASUR, PAKISTAN**

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Water is the medium of life. It is the most abundant compound on earth that is used for drinking purposes. The objective of this research work was to analyze the microbial risk assessment of drinking water quality of Kasur, Pakistan, in terms of total coliforms. Additionally, the presence of pathogenic strains like *Escherichia coli* O157, *Salmonella*, *Shigella*, *Vibrio*, *Acinetobacter*, *Citrobacter* and *Pseudomonas* was also evaluated. They were characterized on the basis of API 20E strip and molecular methods. The sequences of all the strains were also submitted to NCBI Genbank and accession numbers were obtained. All the *E. coli* strains were screened for their ability

not to ferment sorbitol. This non-sorbitol fermenter strain was then confirmed using Prolex<sup>TM</sup> Latex agglutination test for the detection of *E. coli* O157. Isolation of coliforms of fecal origin and detection of *E. coli* O157 in particular proved that our water supplies are not sufficiently protected and disinfected and thus poses potential health threats in the form of diarrhea and dysentery, to the local population. The antibiotic sensitivity pattern of selected bacterial strains was also determined according to the CLSI guidelines 2013. Due to the risk of contamination of drinking water with chromium released by leather tanneries in Kasur, the chromium resistance ability of selected bacterial strains was also determined and it is found that most of the strains are chromium resistant. The ability of biofilm formation of selected bacterial strains in monocultures, co-cultures and multiple cultures was also studied. The effect of iron and chromium on the biofilm forming ability of the strains was also determined. These results suggest that considering water as a low nutrient environment, entry of iron due to the corrosion from pipes adds significant rise in biofilm growth. Thus, elimination of these nutrients from our water system can prove to be an effective strategy towards improved water quality.

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#### SEROPREVALENCE OF HEPATITIS C INFECTION AND ASSOCIATED RISK FACTORS IN HUMAN POPULATION OF TEHSIL KABAL SWAT

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The present study was conducted from 1<sup>st</sup> march to 30<sup>th</sup> July 2015 (Experimental data) and from January 2014 to December 2014 (Secondary data) in Tehsil Kabal Swat to find out the seroprevalence of HCV infection in general population of the area. The rate of prevalence was found higher in male's participants (7.45%) than females (5.34%). Prevalence on the basis of age group was found higher in mature males (8.57%) as compared to the young (6.67%) and old aged males (7.1%). In case of females the rate of prevalence of hepatitis C infection was higher (5.68%) in old females as compared to mature females (5.03%) and young females (5.17%). Month-wise prevalence was found higher in the month June (9.52%), followed by May (7.27%), then by the month of April (5.2%). Prevalence on the basis of the type of house was found higher (10%) in the residents of non-cemented houses, while it was only 4.4% in the residents of the cemented houses. Prevalence on the basis of economic status was found higher in the lower class (55.6%), followed by the middle class (33.3%), while the least prevalence was found in the upper class (11.1%). The prevalence rate was found higher in illiterate people (44.4%), followed by primary level of education (33.3%), which is followed by the participants having secondary level of education (22.2%). The prevalence was found higher in married group (44.4%), followed by single and by widow (22.2%), while the least prevalence rate was found in divorced group of population (11.1%). The highest prevalence rate (66.7%) was found in those individuals who do not possess the history of the disease in their families. It was a clear indication that the percentage of previous infection in males was higher in males (20%). The most dominant symptoms of HCV according to our study was fatigue (33.3%), followed by weight loss (22.2%). The rate of incidence was found higher in the months of July (10.93%). On the basis of gender, the incidence rate was found higher in males (6.34%) as compared to the females (5.84%). Age-wise incidence was reported highest (8.1%) in the mature males, while in case of females the incidence rate was highest in old females (5.6%).

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**OSMOLARITY EFFECT ON THE PRODUCTION OF BIOELECTRICITY BY MIXED MICROBIAL CULTURE**

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Osmolarity in fermentation is vital in determination of the best growth of the microbes leading toward product formation. In this study vegetable residue has been analyzed as substrate for bioelectricity production in locally designed U-shaped microbial fuel cell subjected to different concentrations of NaCl *i.e.* MFC-1 (25 mM), MFC-2 (50 mM), MFC-3 (75 mM), MFC-4 (100 mM), MFC-5 (125 mM) and MFC-6 (150 mM) to fix the suitable osmolarity of the biofuel cell. It was found that the MFC with 50 mM NaCl concentration showed improved response and gave 88.9 W/m<sup>2</sup> power densities and 569 mV of open circuit voltage. The lower range treatment *i.e.* from 25 mM to 75 mM have lower internal resistance when compared to the results obtained from the MFCs with treated with the concentration from 100 mM to 150mM salt. The results showed that NaCl, at its optimum level, play an important role in increasing the bioelectricity and sustaining the internal resistivity.

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## SECTION – I I

### PESTS AND PEST CONTROL

#### DETERMINATION OF SUGAR AND ACIDITY BY CHEMICAL ANALYSIS AS ATTRACTANT SOURCE FOR FRUIT FLY OF MANGO

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Mango fruit (*Mangifera indica* L) is called as “King of the Fruits,” produced in about 90 countries of the world. Pakistan is the 5<sup>th</sup> largest mango producer with production of around one million tons per year. The mango fruit of the world, facing some pre-harvesting pest problems like Fruit fly. To perceive the sensitivity of four different varieties (Sindhri, Chunsu, Beganpali and Sonaro) of mango as a source of attractiveness of fruit fly two bio-chemical characteristic like sugar and acidity of the four mango varieties were examined. We took sample 5gms blended pulp of each varieties for the detection of sugar and acidity of all four varieties, added 1ml of 6N HCl and 40-50ml distilled water, Boiled It (5minutes) for inversion. Neutralized by 7 drops of 40% NaOH and made up volume 100 ml with distilled water. Benedict's solution add 40-50 ml distilled water and boil. Titrate sample solution (in burette) with benedict's solution till color of benedict's solution change to water and white color ppt formed. The obtained results revealed that Sonaro variety (total sugar 0.66gms in 5gms pulp and acidity 0.15%), chunsu (total sugar in 5gms pulp 0.8gms and acidity 0.06%), Beganpali (total sugar in 5gms pulp 0.72gms and acidity 0.13%) and Sindhri (total sugar in 5gms pulp 0.742gms and 0.12% acidity). It was detected that the highest level of reducing sugar in chunsu (total sugar in 5gms pulp 0.8gms and lowest acidity 0.06%) and the lowest reducing sugar in sonaro variety (total sugar in 5gms pulp is 0.66gms and highest acidity 0.15% acidity of sonaro variety).

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#### VARIETAL RESISTANCE OF MAIZE VARIETIES AGAINST RED FLOUR BEETLES (*TRIBOLIUM. CASTENEUM*) COLEOPTERA: TENEBRIONIDAE

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Maize is being the highest yielding cereal crop in the world. Pakistan is the 3<sup>rd</sup> largest maize producer with production after wheat and rice. Maize has prospective to play major role in nutrition of Pakistani people. *Tribolium casteneum* (Tenebrionidae: Coleoptera) is a cosmopolitan pest of many stored products. It cause a bundle of damage to the stored commodities. During present studies we have introduced red flour beetles in two varieties of maize Neelam and Popcorn for identifying the most susceptible variety and observe the comparison of damage on both varieties during laboratory experiments, took the 100gms of each varieties and reared under controlled conditions at 27°C±2°C temperature, 60%-65% Rh and 10 hrs. Photoperiod. During present study it is observed that maximum consumption of red flour beetle on Neelam variety and minimum on

popcorn, 45% and 2% respectively. Present study revealed that Neelam variety of maize is most susceptible variety as compared to Popcorn.

**EFFECT OF DIFFERENT JUJUBE (*ZIZIPHUS JUJUBA*) VARIETIES ON THE LIFE HISTORY PARAMETERS OF *BACTROCERA ZONATA* (SAUNDERS) UNDER LABORATORY CONDITIONS**

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Most tropical fruit flies lay eggs into their favorite fruit, but nothing is known about the link between adult oviposition, preference and offspring performance on different varieties of Jujube. In this study, we examined the influence of four Jujube varieties namely, Gola, Black Gola, Lemon Gola and Achro on the ovipositional preference and performance of the Peach Fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) under laboratory conditions. We found significantly reduced ( $p < 0.05$ ) larval and pupal duration on Black Gola Jujube variety as compared to all tested varieties. However, significantly increased larval period was observed in Lemon Gola. Furthermore, statistically higher ( $p < 0.05$ ) pupal recovery was observed in the Black Gola as compared Achro, Gola and Lemon Gola. Furthermore, maximum number of the males was examined in Achro and maximum female recovery was recorded in Lemon Gola. Our study established that Lemon Gola variety exhibited lower pupal recovery among all the tested varieties. These results could be helpful for the management of fruit flies in different varieties of Jujube.

**ALLELOPATHIC EFFECTS OF *AZADIRACHTA INDICA* (NEEM) AND *EUCALYPTUS CAMALDULENSIS* (EUCALYPTUS) AQUEOUS EXTRACTS ON DIVERSITY OF FOLIAGE SPIDERS' FAUNA AMONG CITRUS ORCHARD (KINNOW)**

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Bio-pesticides are less hazardous than chemically synthesized pesticides but they also have inhibitory nature against plants and animals due to their allelopathic effects. Spiders in citrus orchard act as bio-control agents; they can reduce the pest population up to economic threshold level (ETL). So, the present study was designed to record the allelopathic effects of aqueous extracts of neem and eucalyptus leaves on the diversity of foliage spider fauna among citrus orchard. After completing the whole research trails, total 6788 specimen were collected from three fields. Maximum relative abundance and level of population dynamics was recorded in control field 37.55% (2594), followed by neem extracts treated orchard 34.89% (2368) and then eucalyptus treated orchard 27.56% (1871). From entire sampling and amongst the three fields; maximum diversity was recorded among eucalyptus treated plants (1.8143), followed by neem treated plants (0.5920) and then control (0.0531). Diversity<sub>maximum</sub> ( $H'_{max}$ ) was highest from control (3.4023), following neem (3.3744) and then from eucalyptus (3.2686). Maximum value of evenness (E) was

recorded from eucalyptus (0.5551), followed by neem treatments (0.1754) and then from control group (0.0156). Maximum value of dominance (D) was recorded for neem (1.1754), followed by control (0.9844) and then eucalyptus (0.4449). Richness (R) was recorded utmost from control group (20.5041); while eucalyptus and neem extract treated orchards were with similar values (15.4741). Analysis of Variance (ANOVA) showed non-significant results towards most vulnerable issues ( $F = 0.32$ ;  $P\text{-value} = 0.7291$ ). Kruskal-Wallis Test by Ranks Order results were also non-significant ( $F = 0.10$ ;  $P\text{-value} = 0.9076$ ). Wilcoxon Rank Sum Test results were non-significant (Control vs Neem " $P = 0.8515$ "; Control vs Eucalyptus " $P = 0.7669$ "; Neem vs Eucalyptus " $P = 0.6851$ ") depicting that these practices reduced the population abundance for a while instead of huge damage.

#### **SUSCEPTIBILITY OF SOME SPICES AGAINST (CIGARETTE BEETLE) *LASIODERMA SERRICORNE* (ANOBIIDEA: COLEOPTERA)**

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*Lasioderma Serricorne*, commonly known as the cigarette beetle or tobacco Beetle is a common stored products pest and has been given its name as it also infests tobacco products. Culture of Cigarette beetle was maintained on four types of spices (Coriander, Caraway, red chilies powder and whole chili) obtained from the local market of Hyderabad reared at  $27 \pm 2^\circ\text{C}$ ,  $60 \pm 5\%$  relative humidity and ten hours photo period. Thirty gram of each spices were taken in plastic jars covered with muslin cloth tied with rubber band. Twenty newly emerged adult were introduced in each plastic jar and data collected weekly. During present study it is observed that highest consumption was recorded in red chilies powder and caraway 40% respectively, while lowest recorded in coriander and whole chilies 27% and 23% respectively. Present study reported that infestation of cigarette beetle recorded among in all spices, although cigarette beetle are pest of tobacco.

#### **DETERMINATION OF EXTENT OF PREDATION IN SOME SELECTED CARABID SPECIES FROM CEREAL CROPS OF DISTRICT FAISALABAD**

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The importance of cereal crops can never be under-estimated to support the increasing population worldwide. Aphids are the major pests of cereal crops rendering heavy economic losses. The most effective and environment friendly way to decrease these economic losses is the use of predators. Carabidae is a predator family of order Coleoptera which control aphids by consuming them. The present study was aimed to determine extent of predation of three selected species of family Carabidae namely, *Agonum dorsale*, *Pterostichus cupreus* and *P. melanaius* on three aphid species *Metopolophium dirhorum*, *Sitobion avenae* and *Rhopalosiphum padi* from wheat crops of



district Faisalabad. Experiments were carried out under almost same environmental conditions like field at temperature of  $25\pm5^{\circ}\text{C}$ ,  $60\pm5\%$  RH, and a light regime of 12hL: 12hD. Each of the predator was provided with all the three prey species separately thus a total of nine experiments each with four replicates were conducted in laboratory. Each predator adult and larva of carabid was fed aphid prey species at different densities (20, 30, 40 and 50) placed in petri dishes 9 cm in diameter and 2 cm deep, lined with a moistened filter paper. Fresh aphids were added daily and unconsumed number of aphids and duration of larval instar was noted. All the predators showed maximum prey consumption at initial prey density (20 and 30). Predation rate of each predator species increased with the increase in prey density. The average predation rate for carabids ranged from  $6.00\pm0.41$  to  $39.00\pm0.41$  aphids per day. Predatory efficiency of 3<sup>rd</sup> larval instar was highest than other two larval instar and adults. *M. dirhodum* was preferred prey for most of the predators. Lowest aphid consumption was of *R. padi*. Shortest duration of larval instar was recorded for *A. dorsale* (38 days) and longest for *P. melanarius* (61 days). *A. dorsale* and *P. melanarius* had a significantly greater predation rate as compared to *P. cupreus*. Greater survival period was showed by predators on water ( $32.33\pm0.82$ ) as compared to honey and sugar solution. *Agonum dorsale* had higher survival period on water than other two carabid species. Data was analyzed by analysis of variance (ANOVA) and significant results were observed. Such type of studies are very effective in designing biological control programs to control pests in field and also for integrated pest management programs.

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**MORPHOLOGICAL RESPONSE OF BITTER GOURD (*MOMORDICA CHARANTIA* L.) VARIETIES TO INFESTATION OF MELON FRUIT FLY *BACTROCERA CUCURBITAE* (COQUILLETT)**

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The undertaken research consisted of field and laboratory experiments to investigate the morphological basis of resistance in fruit of bitter gourd (*Momordica charantia* L.), to melon fruit fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Bitter gourd varieties come in various shapes and sizes having fruit surface warty covered with pointed triangular teeth and ridges. Two bitter gourd varieties Pali (narrower shape with pointed ends) and Hybrid (oblong with bluntly tapering ends) were used for experimentation. The field studies included screening of both varieties by field observations on infestation (%), along with larvae, pupae and adults emergence in laboratory, and morphological basis of resistance. The field tolerance or susceptibility of bitter-gourd was further confirmed in laboratory under caged conditions with artificial releasing of 10-20 pairs of fruit flies based on no choice and free choice oviposition trials. Preference and non-preference by fly for oviposition were confirmed by exposing bitter gourd to fruit flies for 1 day, 2 days and 3 days. The variety Pali showed significant field tolerance to fruit flies compared to Hybrid that was highly susceptible. There were significant differences in test varieties for fruit infestation, and larval, pupal and adults densities per fruit. Melon fruit fly field infestation was significantly lower (10%) in Pali compared to 18% infestation in the susceptible Hybrid in both no choice and free choice oviposition trials. Fruit attributes length (cm), width (cm) and number of teeth/ cm<sup>2</sup> showed a positive association with fruit fly infestation and noted the important biophysical fruit traits contributing antixenosis in bitter gourd against pest. There were significant differences in test varieties for fruit infestation and pest multiplication when bitter gourd was

exposed to fruit flies for 1 day, 2 days and 3 days having a positive association with pest density and increase in exposure time. The variety Pali was identified as tolerant source to melon fruit fly under field and laboratory conditions, and can be further used in resistance breeding programs to have a diversify basis of resistance for evolving tolerant varieties against fruit fly *B. cucurbitae*.

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**REVISION AND NEW RECORD OF (HEMIPTERA: PENTATOMIDAE) DISTRICT HYDERABAD AND ADJOINING AREAS**

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Pentatomidae is one of largest families of order Hemiptera. It is famous with the name of stink bugs because they produce bad smell. They constitute an economically important group of hemiptera pests as most of species are phytophagous both nymphs and adults have sucking and piercing type of mouth parts. They suck the sap by piercing their rostrum into plant tissues and lower its vitality from plants they also transmit bacterial, viral and fungal diseases. In the result of extensive survey a total of 363 specimens were collected from March to July 2015, with the help of insect net, light trap and handpicking method from localities Jamshoro, Matiari, Tando Jam, Tando Mohammad Khan from district Hyderabad and its adjoining areas. All collection was preserved according to standard method and identification of genus and species, by using taxonomic keys and literature on the basis of morphological characters. Two species were identified belong to genus *Brachynema*, *B. virens*, and *Coenus*, *C. delius*. Identification was done by using binocular on the basis of morphological description along with measurement of different body and compares the species with the relevant. These species are newly identified in Hyderabad.

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**SUSCEPTIBILITY OF CHICKPEA VARIETIES AGAINST (COWPEA WEEVIL) *CALLOSOBRUCHUS MACULATUS* (CHRYSEMELIDAE: COLEOPTERA)**

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Cowpea weevil is the principal post-harvest pest of chickpea and other stored pulses. It causes severe infestation every year in stored varieties of Chickpea. Culture of cowpea weevil was maintained on four varieties of chickpea (sindhi, sathri, kabuli and kala channa) obtained from local market of Hyderabad reared at 27±2°C and 60±5% relative humidity and ten hours photoperiod. Hundred gram of each chickpea variety was introduced in plastic jars covered with muslin cloth tied with rubber band. Twenty newly emerged adults were introduced in each plastic jar and data collected weekly. During present study it was observed that highest consumption was recorded in sindhi channa 27% while lowest recorded in sathri, kabuli and kala channa 15%, 12% and 8% respectively. Present study revealed that kabuli channa variety found most resistant variety among all four studied varieties of chickpea against Cowpea Weevil.

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**ESTIMATION OF EXTENT OF FORAGING OF *HELICOVERPA ARMIGERA*  
(LEPIDOPTERAN: NOCTUIDAE) ON FOUR DIFFERENT CROPS IN DISTRICT  
FAISALABAD**

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Major constraints to cereal production are stem borers. These are responsible for 5-73% loss in different agro-ecosystems and thus are pests of economic importance. Quantitative analysis of consumption and utilization of host plants by insect herbivores is a commonly used tool in studies of plant-insect interactions. Tomato worm, or Cotton bollworm, *Helicoverpa armigera* is a highly destructive polyphagous pest causing severe loss to a lot of cultivated and uncultivated plant species. Host plant preference of this pest was studied with respect to the consumption rates of their larvae on four host plants viz. brassica, cabbage, cauliflower and turnip and effects of consumption rate on their pupal weights and percent adult emergence. Larval duration was observed to be of 11 days on brassica and turnip and 17 days on cabbage and cauliflower. Maximum foraging was observed on 14<sup>th</sup> day on cauliflower 1.82±0.08g and on 15<sup>th</sup> day of larval development on cabbage and it was 1.47±0.09g. Maximum consumption was recorded on cauliflower 18.04±0.68g and least on turnip 6.47±0.19g. Maximum pupal weight was also observed when larvae were given cauliflower (0.48±0.04g), followed by cabbage (0.45±0.02g), brassica (0.41±0.02g) and least when larvae were given turnip (0.39±0.03g). Strong correlation was observed between pupal weight and adult emergence as more healthy pupae of cauliflower showed maximum emergence followed by cabbage, brassica and turnip. Analysis of Variance (ANOVA) results showed significant differences among total consumption and per day consumption on all the four crops. Studies on the effect of food on the biology of insects are of particular importance in understanding host suitability of plant infesting species and evaluating the magnitude of injury to the crops attacked by them. This may help accordingly, in designing more economic control strategies.

**COMPARISON OF DIFFERENT BOTANICALS FOR CONTROL OF APHIDS ON  
CANOLA (*BRASSICA NAPUS*)**

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Aphid is the main menace for oilseed brassica (*Brassica napus*) crop all over the world. The objective of this study was to compare the effect of different plant extracts viz. aak (*Calotropis procera*), chrysanthemum (*Chrysanthemum indicum*), garlic (*Alium sativum*) and knair (*Thevetia peruviana*) on aphids population in canola under field conditions. The two percent crude extract of each extract was applied. The results showed that aak gave the best results with 55.3% reduction in aphid population followed by chrysanthemum (49.5%) while garlic and knair were least effective with mortality of 39.4% and knair 28.6% as compared to control. The plots treated with aak also gave maximum yield of 2226 kg/ha followed by chrysanthemum (1913 kg/ha). Although garlic and

knair were found least effective but they produced significant yield of 1733kg/ha and 1586 kg/ha, respectively as compared to the yield of control (1266kg/ha) treatment.

#### FEEDING EFFICIENCY OF *CHRSOPERLA CARNEA* AND *COCCINELLA SEPTUMPUNCTATA* ON TWO HOSTS UNDER LABORATORY CONDITION

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The biological control is an important component of integrated pest management. It involves the control of pest population by natural enemies. Red cotton bug (*Dysdercus koenigii*) and dusky cotton bug (*Oxycarenus laetus*) are important pest and cause staining problem. So biological control is the best way of management. In present study predating potential of *Chrsoperla carnea* and *Coccinella septumpunctata* was compared on two pest or hosts under laboratory condition. Experimental layout was designed according to completely randomized design CRD. In each petri dish, eggs, 1<sup>st</sup> and 2<sup>nd</sup> instars of both pests were given (10 in numbers) to all stages of both predators (singly). Result showed that *C. carnea* significantly feed more on eggs of *D. koenigii* i.e., 19.80±0.90 eggs per day, as compared to feeding of *C. septumpunctata* i.e. 13.30±1.13 eggs per day on the same host. Similarly, in case of *O. laetus* eggs, *C. carnea* was found more significantly more effective in consuming more eggs i.e. 14.80±0.49 eggs per day as compared to *C. septumpunctata* which consumed 9.50±0.52 eggs per day. Analogous results were found in case of *O. laetus* 1<sup>st</sup> instar feeding, *C. carnea* significantly feed more on *O. laetus* 1<sup>st</sup> instar i.e. 30.10±1.05 nymphs per day, as compared to *C. septumpunctata* i.e. 14.50±0.76 nymphs per day on the same host. Likewise, *O. laetus* 2<sup>nd</sup> instar feeding showed similar results i.e. *C. carnea* was found statistically more effective as compared to *C. septumpunctata*, 26.10±1.19 and 11.10±0.53 nymphs per day, respectively. Result concluded that *C. carnea* found best predators on both pest i.e. *D. koenigii* and *O. laetus* than the *C. septumpunctata*.

#### SPATIO-TEMPORAL DISTRIBUTION OF FAMILY SYRPHIDAE IN CROP SYSTEM OF FAISALABAD

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One of the most diverse family of Order Diptera is Syrphidae. The predatory effectiveness of hoverflies increases by the ability of hoverflies to detect aphids and oviposit near aphid colonies. Family syrphidae belonging to two sub-families, 10 genera and 13 species *Episyrphus balteatus*, *Melanostoma scalare*, *Melanostoma mellinum*, *Syrphostorvus*, *Sphaerophoria scripta*, *Eupeodes corollae*, *Ischiodon scutellaris*, *Meliscaeva auricollis*, *Meliscaeva cinctella*, *Eristalis aeneus*, *Eristalis tenax*, *Helophilus pendulus* and *Helophilus fasciatus* were sampled from cropland of Faisalabad. From 2218 sampled specimens, total 13 species from fodder, 12 were

sampled from *Brassica*, Eleven species from both vegetables and wheat fields. Largest sample 66.6% of the total samples of syrphids was collected in spring season followed by winter 23.2%, fall 6.80% and summer 3.38% from aerial portions of crops by sweep nets. *E. balteatus* was the most dominant species with 33.4% specimens and showed its distribution round the year with specimens of 23.17%, 7.12%, 1.98% and 1.13% in spring, winter, fall and summer respectively. *M. scalare* was abundant only in two seasons; 4.87% in spring and 4.28% in winter. Maximum diversity  $H'$  was calculated for autumn followed by winter, spring and summer by Shannon-diversity index. Significant results were observed by applying t-test among summer and other seasons, also autumn and winter with spring season. By Canonical Correspondence Analysis it was determined that, most of syrphid species showed positive association with all four crops. Also association was found by various syrphid species with environmental factors like Temperature, Rainfall, Relative humidity and Wind speed indirectly affects the seasonal dynamics of syrphids.

#### INFLUENCE OF FOOD SUPPLEMENTATION ON THE FITNESS OF EGG PARASITOID *TRICHOGRAMMA CHILONIS* (Ishii)

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The role of different artificial diets (honey, sugar, glucose, protein hydrolyzate and egg yolk) and floral nectars (marigold and rose flower) was assessed on the percent parasitism, percent emergence and sex ratio of *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) on the eggs of *Sitotroga cerealella* in Bio-control laboratory of Nuclear Institute of Agriculture, Tando Jam during 2014. The results revealed that females fed on honey and sugar parasitized significantly more host eggs with percent parasitism of 86.60% and 80.60%, respectively than females that fed on other artificial diets and floral nectars. *T. chilonis* feeding on glucose, protein hydrolyzate, egg yolk, marigold and rose flower significantly increased percent parasitism over females that fed on water. The maximum percent emergence of off springs was observed when adults were fed on honey (93.20%) and the minimum was observed in case of egg yolk (69.60%) compared to water (66.20%). However, different artificial diets and floral nectars had non-significant effect on the sex ratio of *T. chilonis*. It was concluded that provision of honey as food supplement to *T. chilonis* adults prior to release would enhance their activity as biological control agent.

#### INSECTICIDAL ACTIVITY OF SOME BOTANICALS AGAINST THE SUCKING INSECT PEST OF COTTON

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Field experiment was conducted during kharif, 2015 at experimental area of Nuclear Institute of Agriculture, Tando Jam to determine the efficacy of seven botanicals viz., peppermint (*Mentha piperita*), turmeric (*Curcuma longa*), hot pepper (*Capsicum annum*), ginger (*Zingiber officinale*),

akk (*Calotropis procera*), bitter gourd (*Momordica charantia*) and mehandi (*Lawsonia inermis*) against the sucking insect pests (jassid, thrips and whitefly) of cotton. Eight treatments including control were maintained following RCBD with three replications. The botanicals were sprayed twice when the population of sucking insect pests reached economical threshold level. Data were recorded at 24, 72 and 168 hours after each application of treatments. The results revealed that extracts of ginger were most efficient bringing about highest percentage mortality (83.33%) in the population of jassids followed by hot pepper (78.33%) and bitter gourd (68.25%). Similar effect of these extracts was found against thrips and whitefly. However, turmeric and peppermint were found to be least effective although both produced significant reduction in the population of target pests as compared to control. Higher yield was also recorded in plots treated with ginger (2304.4 kg ha<sup>-1</sup>), hot pepper (2082.2 kg ha<sup>-1</sup>) and bitter gourd (2000 kg ha<sup>-1</sup>) as compared to that in control (1302.2 kg ha<sup>-1</sup>). It is concluded that all the used botanicals were effective to reduce the population of jassid, thrips and whitefly on cotton; however, ginger and hot pepper performed well as compared to others.

#### EFFICACY OF FIPRONIL AND CHLORPYRIFOS AS BARRIER TREATMENT FOR BUILDING PROTECTION FROM SUBTERRANEAN TERMITES

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The present studies were conducted to determine the barrier efficacy of different doses of chlorpyrifos and fipronil in same depth of soil and same doses of termiticides in different depth of soil against termites in laboratory and fields conditions to check foraging activity of *Odontotermes obesus* (Ramb.) (Termitidae: Isoptera). Two different types of bioassays were performed under laboratory conditions. Collection of termite was carried out from Campus and Post Agriculture Research Station (PARS) area of University of Agriculture, Faisalabad. Treated soil cores of 5cm with four doses (0.05, 0.1, 0.25 and 0.5%) of chlorpyrifos and fipronil were spread in plastic trays. A food source and 100 termites' workers were released to ascertain mortality, gallery formation and repellency. The maximum mortality was in fipronil treatment followed by chlorpyrifos and control after 6, 9 and 12 days, respectively. Gallery formation was maximum in control treatment followed by chlorpyrifos and fipronil after 6, 9 and 12 days respectively. Repellency was non-significant among treatments. In second bioassay, four sizes of soil cores, 2, 4, 6 and 8 cm treated with 0.5% doses of chlorpyrifos and fipronil were laid out in plastic trays in above fashion. Maximum mortality was in fipronil treatment followed by chlorpyrifos and control after 6, 9 and 12 days, respectively. Gallery formation was maximum in control treatment followed by chlorpyrifos and fipronil after 6, 9 and 12 days respectively. Repellency was non-significant. In first field experiment, heavy termites' infested land was selected and divided in twenty five equal sub plots. These sub plots were dug up to 15 cm and soil was treated with four doses (0.05, 0.1, 0.25 and 0.5%) of chlorpyrifos and fipronil and the soil was replaced into these sub plots. Small chunks of poplar (*Populus deltoides*) were put on surface of soils. The maximum weight loss was in control treatment followed by chlorpyrifos and fipronil after 2, 4 and 8 weeks respectively. In second field experiment, another field plot with heavy termites' infestation was divided in 28 equal sub plots and these sub plots were dug up to 15, 30, 45 and 60 cm and the soil was treated with same dose (0.5%) of chlorpyrifos and fipronil and soil was replaced in to these sub plots with chinks of poplar as mentioned above. The maximum weight loss was in control treatment followed by chlorpyrifos

and fipronil after 2, 4 and 8 weeks respectively. In nutshell, fipronil caused significantly high mortality as compared to chlorpyrifos, however, galleries were shorter in fipronil than chlorpyrifos. Percent weight loss of wood was maximum in chlorpyrifos and minimum in fipronil. Thus fipronil can be used in building foundations as barrier treatment.

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#### ANTITERMITE ACTIVITIES OF BARK, SAP- AND HEARTWOOD EXTRACTIVES OF *ALSTONIA SCHOLARIS* L.

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Extractives of the bark, sap and heartwoods of *Alstonia scholaris* L. were investigated on termites' mortality, repellency and difference of gallery formation in acetone, methanol, petroleum ether and distilled water solvents. 10, 20, and 30% concentrations of each wood extractive were prepared. For the mortality bioassay twenty grams of soil was taken in the petri dishes and then was treated with each concentration of sap, bark and heartwood extractives; mortality data was taken after every 2 hours up to 12 hours and then 12 hours until all the individuals died. For the repellency test Whatman's Filter paper No. 42 was divided into two halves, one half was treated with each concentration of extractive while the other half was treated with the solvent only and repellency was monitored for one hour after every ten minutes. The effect of extractives on the gallery formation by the termites was also studied. For this purpose 20 grams soil was taken in the petri dishes and divided into two halves. One half was treated with each concentration of extractive and other half was treated with solvent only. Length of galleries were measured after every 15 minutes up to 60 minutes. All these experiments were performed in Completely Randomized Design (CRD) in laboratory at 28±2°C. Each time a new set of termite workers was used. Data obtained from all these experiments was subjected to minitab16 for statistical analysis by using General Linear Model. The results obtained from study showed that in first bioassay of mortality; maximum percent mortality was in acetone based heart wood extractive followed by petroleum ether, methanol and distilled water based heart wood extractives at 30 % concentration after 2, 4, 6, 8, 10, 12, 24 and 36 hours respectively. In second bioassay of gallery formation; maximum gallery formation was in control treatment followed by methanol, petroleum ether and acetone based heart wood extractive at 30% concentration after 15, 30, 45 and 60 minutes of exposure respectively. In third experiment of repellency; maximum percent repellency was in Acetone based heart wood extractive followed by methanol, petroleum ether and distilled water based heart wood extractive after 10, 20, 30, 40, 50 and 60 minutes of exposure respectively.

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#### EVALUATION OF COMMERCIAL RESINS AS WOOD PRESERVATIVES AGAINST SUBTERRANEAN TERMITES

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Field experiments were undertaken to evaluate the protective potential of three commercial resins viz., P3133, P3118 and P3109, obtained from Descon Chemicals Lahore, on *Ficus religiosa*

(peepal), *Pinus wallichiana* (kail) and *Populus deltoides* (poplar) woods when exposed to underground pits having a base of concrete with pegs in contact with soil to make access of termites *Odontotermes obesus* (Ramb.) (Termitidae: Isoptera). These concentrations (10, 20, 30 %) of each compound were applied by dipping (6 & 12 hours) and brushing (2 and 4 coatings) and treated woods were placed in above said arena at Post Graduate Agriculture Research Station, (PARS), University of Agriculture, Faisalabad for a period of 4 and 8 weeks. After exposure, the wood stakes were brought to the laboratory cleaned carefully to remove the soil. Final weight of wooden stakes was measured by using electronic balance after drying in an oven. The weight loss percentage of each wood was calculated. In all these experiments, treatments were laid out in the experimental units by using Randomized Complete Block Design (RCBD) in factorial layout. Data were analyzed by using suitable statistical techniques. Results showed that 30% of each resin compound performed excellent by providing maximum protection to woods as compared to other concentrations in two methods of application. Dipping of wooden stakes in resins for 12 hours showed significant difference from other methods of applications. Temperature treatment of 200°C and moisture below 10% before application of resins was found to be significantly best in protecting the woods. Resin types and their combinations have shown high potential of protecting the woods from *O. obesus* infestation. Commercial resins left no stain on woods and thus can be good substitute of chemicals for protecting the woods from termites attack.

**TOXICITY OF DIFFERENT INSECTICIDES AGAINST COTTON MEALYBUG,  
*PHENACOCCLUS SOLENOPSIS* (TINSLEY) UNDER COTTON, *GOSSYPIMUM HIRSUTUM*  
(L) FIELD CONDITIONS**

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The cotton mealybug, *Phenacoccus solenopsis* (Tinsley) is known as most vulnerable sucking insect pest of cotton crop which found on different alternate host plants of different genera. Due to mealy wax protection the insecticides found lesser effective. The different kinds of insecticides either rational or irrational were applied previously but no one found to be sever effective. Consequently, novel insecticides provided the different formulations to diminish this pest. Therefore, the insecticides namely; Acetamaprid (Mospilan) 20 SP, Pyriproxyfen (Admril) 10.8 EC, Diafenthiuron (Polo) 500 SC, Acephate (Safate) 75 SP, Nitenpyram (Amrasca) 10 SL, were applied which replicated four interval times and finally compared with the forbidden insecticides under control plot. Thus data were gathered at pre and post-treatments on 24, 48, 72hrs, 7<sup>th</sup> and 12<sup>th</sup> day after application of insecticides during, 2015 in Kharif season at district Khairpur under cotton field conditions. The data was formulated under Hinderson and Tilton formula. It was observed that these insecticides provided the bit effective on 2<sup>nd</sup> and 3<sup>rd</sup> day after application of insecticides besides no any insecticide permanently reduced the mealybugs population till the last day of data collection. The insecticide namely; Acetamaprid provided better results when compared with the other insecticides which showed the overall mean reduction percent 1.19 in all sprays in comparison of control plot. The other insecticides were found lesser effective like as; Acephate that reduced the overall -10.86% followed by Diafenthiuron -11.98, Nitenpyram -21.74 and Pyriproxyfen -23.59% when compared with control plot 4.76, respectively. The analysis of variance showed the non-significant difference among all tested pesticides at (P<0.05) except



Acetamaprid. It is concluded that the pesticide Acetamaprid provided better results infavour to control the cotton mealybug, thus the other tested insecticides are here by recommended to do not apply for the purpose of control the cotton mealybug and these should be banned and strongly forbidden for their application under cotton mealybug affected crop.

**SURVEILLANCE AND BIOLOGICAL STUDIES OF MANGO LEAF MINER,  
*ACROCERCOPS SYNGRAMMA* (MEYRICK) AT DISTRICT KHAIRPUR**

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Recently in upper Sindh mango leaf miner created major losses on newly emerged leaves of mango plants. So, an experiment on its occurrence and biological parameters were carried out to assess the mango plant losses and their remaining active period of pest on different varieties such as; Chuanca, Fajiri, Sindhri, Siroli and Langra during, 2014. The results showed that significantly ( $P < 0.05$ ) highest damage of *A. syngamma* was recorded on Chuansa, whereas; the lowest on Langra varieties. The pest undertakes four maximum peaks and remained active during emergence of plant new flushes in the month of August - October, then damage declined with slowdown appearance of plant new flushes. The female singly laid eggs 49-59 on Chuanca, 42-59 on Fajiri, 44-56 on Siroli, 38-52 on langra and 39-51 on Sindhri mango plant leaves with an average to 54.8, 49.2, 49.6, 46.0 and 44.2 eggs. Thus, the eggs fertility of *A. syngamma* on Chuanca, Fajiri, Sindhri, Siroli and Langra, 92%, 76%, 90%, 72% and 84%, respectively. The leaf miners completed their biology ranging from 19-30, 19-30, 21-27, 20-30 and 19-33 days on different varieties of mangoes Chuanca, Fajiri, Sindhri, Siroli and Langra under laboratory conditions. The correlation coefficient results of temperature and relative humidity with leaf miner populations on different mango varieties were showed non-significant, negative and positive correlations.

**PREVALENCE AND EMPATHY OF INNUMERABLE BITING LICE SPECIES OF  
DOMESTIC AND POULTRY CHICKENS OF UPPER SINDH – PAKISTAN**

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The study was carried out at different locations of Sukkur region at Taulka level such as; Pano Akil, Rohri and Sukkur on fortnightly interval basis during, 2013-14. Those chickens (n=20) domestic chickens were selected and observed from neck, chest and cloacal regions, who were never been injected or used any drugs for precaution of lice repellent or kill. There were 4 kinds of lice species were found and thus identified from lice specialist. Four species namely; *Menopon gallinae*, *Goniodes disimilis*, *Machaeriaemus clyae* and *Columbicola tschyulyschma* of lice on the chicken bird as a host were reported first time from this Sukkur region. The samples for the further confirmation of species were brought under Entomology laboratory conditions, department of Zoology, SALU – Khairpur. The results further indicated that, the peak population of lice were

observed at taluka Pano Akil in the month of June (35.78) / bird and least population in January (10.26) with the overall mean population (44.75) / bird whereas; in taluka Rohri the peak population was observed in July (11.85) and least in February (3.78) with the overall mean population (33.17) / bird and in taluka Sukkur in June (9.50) and least in January (2.40) with the overall mean population (11.28) / bird, respectively. It was further observed that in the taluka Pano Akil, the overall infection present population of *M. gallinae* was observed (40.22%) / bird followed by *G. dissimilis* (12.94), *M. clyae* (22.93) and *C. tschulyschma* (13.75). In Rohri region, the *M. gallinae* (41.46%) followed by *G. dissimilis* (30.33), *M. clyae* (24.95) and *C. tschulyschma* (12.94). From taluka Sukkur, *M. gallinae* (42.83%) / bird followed by *G. dissimilis* (26.47), *M. clyae* (22.55) and *C. tschulyschma* (13.59), respectively. It was concluded through this research study, that the temperature provided positive effect to enhance the lice parasites whereas; the RH% found to be no any effect for development of the lice on poultry birds. Therefore, it is suggested that, integrated control strategies should be done in place to improve chicken productivity and enhance smallholder livelihood in these areas at Sukkur region.

#### REPELLENCE OF SMOKE OF FIVE INDIGENOUS PLANTS AGAINST *TRIBOLIUM CASTANEUM* (RED FLOUR BEETLE)

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Many indigenous plants have bioactive natural products which can be used for pest control strategies. The economic and environment friendly bio-technologies to protect stored grains from deterioration by insect pests can help in Integrated Pest Management by minimising post-harvest losses. Smoke of burning wood, dried leaves and straw to repel insects is an old age and common practice in villages. The smoke by burning dried plant material can give significant repellent properties against insect pests. In this regard a study on the smoke of five plants, *Azadirachta indica* (neem), *Valeriana officinalis* (valerian), *Acorus calamus* (sweat flag), *Curcuma longa* (turmeric) and *Saussurea lappa* (kuth) was carried out to test their repellence against *Tribolium castaneum*, (red flour beetle). For the trials smoke-proof two-chambered glass cages, with a door divider separating one chamber from the other, were used. Ten days old beetles were released in one chamber where the 5 gm plant powder was completely burnt on ignited charcoal on a metal stand. The divider was opened with a gap of 1 cm for exit of the insects that oriented away from plant smoke into the second chamber. The charcoal repellence was observed by the same procedure. The experiments were replicated five times. Results showed that *Azadirachta indica* (neem) smoke proved the best repellent by giving 75.89±2.14% repellence after 2 hours, which increased up to 95.65±1.58% after 8 hours. *Saussurea lappa* (kuth) was found the second promising repellent by giving 44.15±1.87% repellency which decreased to 41.57±1.46% after 8 hours. *Acorus calamus* (sweat flag) showed 23.15±1.75% repellence which decreased to 15.20±2.77% only, after 8 hours. *Valeriana officinalis* (valerian) was found a weak repellent by showing only 18.40±1.49% repellence which increased up to 26.47±13.50% after 8 hours (LSD<sub>0.05</sub> calculated by Fisher's Least Significant Difference was for time interval: 4.50; for plant concentration: 5.03). In these studies neem was found the most promising and persistent repellent

against *Tribolium castaneum*, (red flour beetle); however this is a preliminary study and requires further trials in the laboratory and produce ware houses to validate the repellent activity of smoke omitted by burning the plant materials. More over there is a need for conservation and awareness about the valuable natural plant products.

**COMPARATIVE EFFICACY OF NOVEL PESTICIDES AGAINST JASSID, *AMRASCA BIGUTTULA BIGUTTULA* (ISHIDA) UNDER COTTON FIELD CROP CONDITIONS**

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The research study was carried out under the cotton crop field conditions at district Khairpur during, 2015 to investigate the efficacy of different insecticides such as; Acetamaprid (Mospilan) 20 SP, Pyriproxyfen (Admril) 10.8 EC, Diafenthion (Polo) 500 SC, Acephate (Safate) 75 SP, Nitenpyram (Amrasca) 10 SL, against jassid a vigorous sucking pest of cotton crop. The above mentioned 5 insecticides / treatments were replicated 4 times whereas; the 6<sup>th</sup> treatment known as control plot was kept as un-sprayed. The data was taken on pre-treatment and the post-treatments; after use of pesticides on 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 7<sup>th</sup> and 12<sup>th</sup> day, respectively. The reduction percent of jassid was evaluated through the Henderson and Tilton formula. Therefore, the all toxicant pesticides reduced the jassid mean percent population up to 12<sup>th</sup> day after application of different pesticides on cotton crop. The results further indicated that the insecticide Nitenpyram was found more effective when compared with less effective insecticide Pyriproxyfen to the *Amrasca biguttula biguttula* (Ishida). The highly toxicant insecticide Nitenpyram showed the overall reduction 68.61 percent in all sprays when compared with the control plot. The second insecticide Acephate reduced the overall 58.75 followed by Acetamaprid 49.41, Diafenthion 27.48 and Pyriproxyfen 23.61 when compared with control plot 1.82 percent. ANOVA showed the significant difference among all tested pesticides at (P<0.05) level. It is concluded from the research study that the pesticide Nitenpyram provided better reduction against the cotton jassid under field conditions, therefore, it is recommended to be applied at per time interval basis.

**POPULATION DYNAMICS OF APHIDS, *LIPAPHUS ERYSIMI* (KALT) AND ITS NATURAL CONTROL ON DIFFERENT OIL SEED MUSTARD VARIETIES**

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The field experiment was carried out in the experimental area of Shah Abdul Latif University, Khairpur during Rabi season, 2014-15 on six cultivated mustard varieties viz., S-9, Toria selection, Aghati sarhein, J.S -13, Janbho selection and Sindh raya. The results indicated that sucking insect pest; *Lipaphis erysimi* (Kalt) appeared after from one month of cultivation the

different mustard varieties till harvest of the crop. The overall means showed that the maximum population was recorded on T. selection, 28.92 per leaf followed by A. sarhein, 22.45; J.S-13, 13.71; S-9, 13.31; S. Raya, 11.55 and J. selection, 10.52, respectively. The analysis of variance showed significant differences in the population of pest in all varieties. The predators' activities were recorded on the varieties having maximum pest activities. Overall data suggested that the population of insect pest and predators remained constant on all varieties. Finally, the less population of the predators was due to less population of aphids. Similarly; the predator population was also observed on same 6 varieties of mustard crop namely; S-9, T. selection, A. sarhein, J.S-13, J. selection and S. raya. The overall means showed that the maximum population of *C. coccinellids* recorded on A. sarhein, 1.36 per plant followed by T. selection, 1.14; S-9, 0.69; J.S- 13, 0.65; S. raya, 0.63, and J. selection, 0.38, respectively. It was concluded that, in starting the predator population was found lesser but when it went in to peak levels the pest aphid population reduced which found to be positive correlation with pest population.

**COMPARISON OF RATIONAL AND IRRATIONAL PESTICIDES AGAINST COTTON MEALYBUG, *PHENACOCCLUS SOLENOPSIS* (TINSLEY) ON COTTON LEAVES UNDER MAINTAINED TEMPERATURES**

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The cotton mealybug, *Phenacoccus solenopsis* (Tinsley) is known as most vulnerable sucking insect pest of cotton crop which found on different alternate host plants of different genera. Therefore, the synthetic and bio-synthetic pesticides namely; Lambda and Neem oil (repellent) were applied to check their effect which were replicated five interval times. Thus data were taken at uniform pre-treatments (100) counted mealybugs and the post-treatments on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day after application of insecticides at Entomology laboratory, department of Zoology, SALU - Khairpur during, 2015 under maintained room temperature 25±2°C and air conditions 18±2°C, respectively. The data were formulated under LDP line (Schneider-Orelli) formula. It was observed that Neem-oil pesticides provided the higher effect on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day after application while, Lambda insecticide little bit reduced the mealybug population up to last day of data collection under room maintained temperature whereas, there was negative effect under air-conditioned temperature. Neem-oil provided the better results under room maintained temperature and air conditioned room temperature by reducing overall mean percent population of mealybugs on 1<sup>st</sup> day (31.60), 2<sup>nd</sup> day (21.56) and 3<sup>rd</sup> day (16.12)%, and (10.76), (10.00), (6.40)%, respectively. When these compared with the Lambda that reduced (1.83), (2.90), (1.59), and (-5.69), (-2.93), and (-1.50) %. The analysis of variance showed the significant difference among the two pesticides at (P<0.05) whereas; the non-significant results were observed among the replicated treatments on different days after application of both pesticides. It is concluded that the pesticide Neem-oil provided better results in favour to control the cotton mealybug on both compared temperatures, thus the other tested insecticide is here by recommended to do not apply for the purpose of control this vigorous cotton pest of cotton and this rational Lambda pesticide should be banned and strongly forbidden for their application.

### INCIDENCE AND DOCUMENTATION OF BUFFALO TICKS AT DISTRICT, KHAIRPUR – PAKISTAN

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The research study was carried out from taluka Gambat, district Khairpur in summer season during, 2015. The data were made in the earlier morning when animals were in rest position from different body parts of buffalo such as: head, thorax, abdomen, udder and tail regions (n=20) buffaloes. The overall mean population of Gambat taluka throughout on the head region was observed (0.55±0.29), thorax (0.41±0.25), abdomen (0.53±0.29), udder (3.48±0.73) and the tail (3.92±0.71). The overall highest mean population was observed (2.19±0.84) / animal during the second fortnight of August, 2015 whereas; the lowest (1.33±0.39) / animal during the month of first fortnight of May, 2015, respectively. The analysis of variance showed the significant difference among the all body parts effected with the ticks throughout the buffalo animal at (P<0.05) whereas; non-significant difference among the dates with the occurrence of ticks on all body parts effected throughout the summer season. However; during the research study we found and identified the few of them six new species of ticks; *Hyalomma anatolicum*; *Hyalomma anatolicum excavatum*; *Hyalomma anatolicum excavatum*; *Hyalomma ssp*; *Hyalomma anatolicum excavatum*; *Hyalomma ixodes excavatum*; *Hyalomma ssp*; *Ixodes ricinus*; *Hyalomma Excavatum*; *Hyalomma ixodes excavatum*, overall result showed highest population of animals tick and new species showed rapidly enhance the index of animals tick, which reduced animals performances, growth and farmers finance.

### APPEARANCE OF LEPIDOPTERAN PEST RICE LEAFFOLDER AS MAJOR PEST OF RICE CROP IN OUTSKIRTS OF INTERIOR SINDH

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The present study describes and reveals the presence of Rice leaffolder as major pest in environs of Sindh. Sindh is an immensely luxuriant agricultural area that contributes number of valuable country crops such as wheat, Rice, Cotton, sugarcane etc and remains viable for invasion of number of insect pests. Insect pests of Rice crop identified worldwide are enormous but in Pakistan previous findings suggest abundance of Rice stemborer as major rice pest whereas new findings in interior Sindh mainly focused in District Kamber Shahdadkot that is rich in rice crop disclose an appearance of lepidopteran pest *Cnaphalocrocis medinalis* (Guenee, 1854) commonly called Rice leaffolder as major pest in this area. Study was sequentially carried out from 2013 to 2014 in months of September and October with sample collection of various rice pest species but with surprisingly low count of rice stemborer in the area and comparatively larger collection of Rice leaffolder. Its identification, statistical evaluation was carried out in entomological laboratory of zoological department.

# **DIET OF SMALL INDIAN MONGOOSE (*HERPESTES JAVANICUS*) INHABITING AGRO-ECOSYSTEM OF DISTRICT SIALKOT**

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Small Indian mongoose (*Herpestes javanicus*) is an important small carnivore, beneficial for farmers in agricultural land since it consumes insects and rodents. The current study aimed to investigate its diet composition in the agro-ecosystem of district Sialkot. Four different sampling sites were selected in the habitat of the species and visited fortnightly for collection of faecal samples for diet analysis. During faecal analysis, the contents were segregated into identifiable groups as either being animal-based or plant-based. Mammalian prey species were identified from the recovered hair by preparing light microscopic slides, while bird species were identified from recovered feathers. Results showed that most common food components of small Indian mongoose were insects and rodents, the species also consumed birds, and plants leaves and seeds). Occasionally it also fed upon snails. The insects' food of the species included five different orders. The mammalian or rodent prey species included *Bendicota bengalensis* (Lesser bandicoot rat), *Nesokia indica* (Short-tailed bandicoot rat), *Suncus murinus* (Asian musk shrew), *Tatera indica* (Indian gerbil) and *Mus musculus* (House mouse). Diet composition of small Indian mongoose varied during different seasons of the year; the consumption of insects was high during summer season as compared to the other three seasons. Prey species richness (S) was high in summer (16), the diversity index (H') was highest during summer season indicating that the diet of the species was more diverse during the summer (2.77) as compared to winter (2.30), autumn (2.20) and spring (2.08) seasons. The evenness index (E) showed slightly higher values in spring (0.95) and autumn (0.95). The study concludes that small Indian mongoose has a diversified diet that includes both animal and plant matter, the animal food is consumed more heavily (58%) than the plant food (8%). The prey species of the small Indian mongoose are dominated by insects (42%), followed by rodents (10 %) and feathers (2%), with seeds (2%) and plant leaves (5%).

## **DEVELOPMENT OF LARVAL DIETS FOR REARING OF *CHRYSOPERLA CARNEA* STEPHENS (NEUROPTERA: CHRYSOPIDAE) UNDER LABORATORY CONDITIONS**

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Green lacewing *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) is an important biocontrol agent of soft bodied insect pests. *C. carnea* larvae predate upon immature stages and adults of aphids, jassids, whitefly and other pests of agricultural crops. Studies were conducted to develop artificial diet for *C. carnea* larvae to improve the mass rearing procedures. Three meridic diets having different protein sources were tested for survival and developmental parameters. It was found that Diet A (Chicken liver Fresh) was better than the Diets B (Freeze dried liver) and the Diet

C (Beef Extract). The larval survival and adult emergence on Diet A was 83 and 75 percent whereas on diet B and diet C was 40 and 50 percent respectively. The larvae passed through all three instars and pupated well. Adult emergence for diet C and diet A was 38 percent and 83 percent whereas, no adult emergence occurred for diet B. The percentage of females on diet C developed larvae was more than those of developed on diet A. Female longevity of larvae developed on Diet C was more than diet A. Percentage of males developed on diets A and C were 84 percent and 65 percent. The average longevity of males on diets A and C was 7 days and 4 days respectively.

**EFFECT OF DIFFERENT INSECTICIDES IN REDUCING GUMMOSIS, ATTRIBUTED TO PEACH FLAT-HEADED BORER, *SPHENOPTERA DADKHANI* OBEN. (COLEOPTERA: BUPRESTIDAE) IN PLUM TREES**

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Studies were conducted to evaluate the efficacy of seven insecticides, *i.e.*, Fyfenon 57EC, Curacron 500EC, Thiodan 35EC, Lorsban 40EC, Regent 5%SC, Ematac 1.9EC, and Confidor 20SC as spray in plum orchard in reducing the gummosis damage induced by Peach flatheaded borer. The mean density of new gum points /m<sup>2</sup> on bark surface area treated with Confidor 20SC @ (30 ml/ 10 L) was significantly lower (7.55) followed by Thiodan 35EC (7.80) applied @ (50 ml/ 10 L), as compared to the untreated control (40.49). In trees that received two applications, the mean density of new gum points/m<sup>2</sup> of bark surface area were significantly lower (11.24) as compared to the trees that were sprayed once (15.86). Highest percent reduction in gummosis was offered by Confidor 20SC (81.4%) followed by Thiodan 35EC (80.7%), whereas the lowest by Curacron 500EC (70.4%). It is suggested that two sprays of Imidacloprid (Confidor 20SC) can successfully reduce gummosis problem caused by this pest.

**PREDATORY POTENTIAL OF *CHRYSOPERLA CARNEA* STEPHENS AGAINST JASSID *AMRASCA DEVASTANS* DIST. AND WHITEFLY *BEMISIA TABACI* GENADIUS ON BRINJAL CULTIVARS IN FIELD CONDITIONS**

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Brinjal (*Solanum Melongena* L.) is an important solanaceous vegetable crop in tropics and sub-tropics, having good source of nutrients, minerals and vitamins so attacked by several insect pests from planting till harvesting. Present field study was conducted against sucking pests by releases egg cards of generalist predator *Chrysoperla carnea*. Inundative releases of *C. carnea* were made weekly, to control sucking insect pests in seven lines of Brinjal. The average infestation of whiteflies and jassids under treated and untreated conditions was significantly different in all seven varieties of Brinjal. It was found that mean number of jassids and whiteflies on Brinjal varieties increased steadily in untreated plot. The average percent infestation of whitefly was 0.4 to 3.5 and 2.5 to 8.2 per leaf in treated and untreated plots. Average number of jassids decreased in the mid of

season in treated plot. It was recorded that minimum percent infestation of jassids was 20.5 and maximum 55.4 in treated and untreated plots, respectively.

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#### IMPACT OF OILS ON THE EFFICACY OF CUE-LURE FOR MALE ANNIHILATION OF MELON FLY

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Fruit flies are the major pest of fruits and vegetables in the country as well as throughout the world causing tremendous losses. Cue-lure attracts a large number of fruit flies attacking various fruits and vegetables. To reduce the cost of male annihilation technique and enhance the attraction of this lure, five oils *i.e.* canola, sun flower, olive, citronella and clove were blended @ 25, 50, and 75 % in Cue-lure as extender in order to see its efficacy in comparison with commercial Cue-lure trap. The trial comprised of five main treatments *i.e.* 5 oils, and 4 sub treatments *i.e.* 4 concentrations. *i.e.* 25%, 50% and 75% of each oil, added in Cue lure. Each mixture of oils and lure had 10% sugar and 5% dipterex as killing agent. 4 ml of test combination /trap was applied on cotton wick. The traps were installed at a height of 2.00 m above ground level in RCB design for 11 weeks in bitter melon crop at Peshawar. Standard Cue-lure as well as traps loaded with only oils was also installed for the comparison of the results. The experimental field was surrounded by more than 60 kanals of sponge melon cultivation, which ensured the availability of flies for evaluating the test materials. Data was recorded weekly for number of flies /trap after bringing dead flies in shoppers to laboratory for counting. The results revealed that none of the oil in pure form was effective for trapping melon fly, however, in 50% canola and sunflower combination with Cue lure captured mean number of 18 and 13.4 flies, respectively as compared to 12.7 flies/ trap/ week in standard Cue-lure trap. In case of citronella oil, 19.8 and 25.3 flies were trapped with 25 and 50% concentration mixed with lure, which were more than the flies captured in standard trap. 75 % Olive oil attracted 12.2 flies and was at par with standard trap. This indicated that addition of oils up to 75% did not affect the efficacy of lure and, hence these oils can be mixed in Cue-lure to reduce the cost male annihilation technology for melon fly.

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#### DISTRIBUTION OF POPULATION AND PARASITISM OF COTTON WHITEFLY (*BEMISIA TABACI* GENN.) IN RELATIONSHIP WITH ABIOTIC FACTORS IN SINDH, PAKISTAN

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Cotton whitefly (*Bemisia tabaci* Genn.) is a devastating pest of cotton crop in Pakistan and often causes economic damage. Spatial distribution pattern of its population and parasitism was studied among fourteen cotton growing districts of Sindh during the months of August and September for two consecutive years *i.e.* 2012 and 2013. For this purpose, a survey of whitefly populations was conducted in Shaheed Benazirabad, Umerkot, Tando Allahyar, Badin, Thatta, Khaipur, Sukkur, Sangar, Nausharo Feroze, Tando Muhammad Khan, Mirpurkhas, Hyderabad,



Matiari and Ghotki districts. There was a significant difference in whitefly population and percent parasitism among the fourteen districts. The maximum whitefly population was recorded in Khaipur, Sukhur, Sangar and Nausharo Feroze while the minimum population was recorded in Hyderabad and Mirpurkhas. On the other hand, the highest percent parasitism was observed in Khaipur, Tando Muhammad Khan, Nausharo Feroze and Sangar whereas, the lowest percent parasitism was recorded in Hyderabad and Matiari. Both the population of whitefly and its percent parasitism had a strong positive relationship with relative humidity while both had only positive relationship with the temperature.

**ASSESSMENT OF *TYLENCHULUS SEMIPENETRANS* POPULATION DENSITY IN DISTRICT SARGODHA AND ITS SYNERGISTIC EFFECT WITH *FUSARIUM* SPECIES IN RELATION TO SLOW DECLINE DISEASE OF CITRUS**

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A field survey was carried out in different citrus grower's field of District Sargodha (Sargodha, Sillanwali, Bhalwal and Kot Momin). Survey results showed that *T. semipenetrans* prevalence was maximum in tehsil Sargodha and then Kot Momin, and minimum in Sillanwali. Maximum population density of *T. semipenetrans* was recorded in April and October, and minimum population was recorded in December. Maximum population density of *T. semipenetrans* was recorded in 20-25 years old plants than 10-20 years old plants while minimum population was recorded in below 10 years old citrus plants. In greenhouse experiment, *T. semipenetrans* was inoculated on different varieties of citrus (Feutrel early, Kinnow, Musambi, Red blood and Shakri). *T. semipenetrans* inoculation reduced the plant growth parameters (number of leaves, plant height, fresh root weight and fresh shoot weight) as compared to control. In second experiment, three species of *Fusarium* (*F. semitectum*, *F. oxysporum* and *F. solani*) were inoculated. *F. solani* significantly reduced more plant growth parameters as compared to other species. In third experiment, *T. semipenetrans* and *Fusarium* spp. were combined inoculated in five citrus varieties. The results revealed that plant growth parameters were highly reduced in combine treatments as compared to single treatment.

**EFFECT OF GAMMA IRRADIATION ON THE MORTALITY AND GROWTH INHIBITION OF MANGO SCALE *ASPIDIOTUS DESTRUCTOR* SIGNORET AND CITRUS SCALE *AONIDIELLA AURANTII* (MASK) (HEMIPTERA: DIASPIDIIDAE)**

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Pakistan stands among the top ten citrus growing countries in the world. Pakistani Kinnow has good demand abroad, as foreign fruit vendors generally prefer it due to its taste and peelable nature. Mango is ranked as second important fruit for export after citrus. Pakistan exports citrus to Russia, Iran and Dubai, (0.3-0.4 million tons) and mango to USA, Australia (0.12million tons) and

has great export potential for mangoes and citrus to developed countries but faces problem due to presence of quarantine pests and WTO constraints. The citrus psylla, *Diaphorina citri* Kuawayama, and citrus red scale *Aonidiella aurantii* are the key pests of citrus in Pakistan and declared as quarantine pest in many parts of the world. Mango scale *Aspidiotus destructor* is quarantine pest of secondary importance. Pakistan consequently loose export of citrus and mangoes to the pest free countries as the immature stages, particularly eggs and nymphs, can be transmitted with fresh fruits to the importing countries. Recently irradiation has been adopted as safe measure for disinfestations of the quarantine pests. Herein we report the disinfestations of citrus and mangoes by the use of irradiation and application of specific doses for the control and growth inhibition of various stages of these pests on fruits prior to their export. Fruits with eggs, first, second stage nymphs and pre-oviposition females of *Aonidiella aurantii* and *Aspidiotus destructor* were exposed to a series of irradiation doses between 100 and 300 Gy to determine the most tolerant stage. The egg stage of *A. destructor* and *A. aurantii* was found as the most susceptible and 3<sup>rd</sup> stage nymphs as the most tolerant stage to irradiation. The pattern of tolerance to irradiation in was eggs < 1st instar < 2nd instar < 3rd instar adults. A dose of 217.7 was determined to completely stop *A. destructor* and *A. aurantii* development to next stages and therefore, should provide quarantine security for citrus and mango scales on exported citrus and mangoes.

**STUDIES ON PARASITISM PREFERENCE OF *DIRHINUS GIFFARDII* (SILV.) ON *BACTROCERA CUCURBITAE* (COQ.) PUPAE REARED ON DIFFERENT HOST VEGETABLES**

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The Melon fruit fly *Bactrocera cucurbitae* (Coquillett) is a serious pest of different vegetables and fruits in Pakistan inflicting economical damages. It has been widely documented that food is positively correlated with the biological parameters of the insects. Therefore an experiment was conducted to determine the parasitism preference of pupal parasitoid *Dirhinus giffardii* on *B.cucurbitae* pupae reared on different vegetables including Bottle gourd (*L. siceraria*), Bitter gourd (*M. charantia*), Ridge gourd (*L. acutangula*), Round gourd (*P. fistulosus*) and Cucumber (*C. Sativus*) under no choice and free choice experiment. Results revealed that significantly higher ( $P < 0.05$ ) parasitization ( $24.8 \pm 0.48$ ) and female ( $12.4 \pm 0.68$ ) were yielded when the pupae of *B.cucurbitae* were reared on Cucumber (*Cucumis sativus*) under no choice test. Similarly maximum number of parasitized pupae ( $10.0 \pm 0.31$ ) and female ( $4.4 \pm 0.24$ ) were recovered from pupae reared on *C. sativus* under free choice test. Furthermore studies showed that pupae of *B.cucurbitae* were more preferred for parasitization when reared on Cucumber (*C. sativus*) followed by Bottle gourd (*L. siceraria*) and Ridge gourd (*L. acutangula*) under no choice test whereas pupae of *B.cucurbitae* less preferred for parasitization when supplied with Ridge gourd (*L. acutangula*) and Bottle gourd (*L. siceraria*) under free choice test.

**PHYTOCHEMICALS: PERCEPTIONS AND ROLE IN AGRO-ECOSYSTEM**

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Concerns have been raised from the present agricultural technology. Pesticides and other chemicals contaminated food, environment and created many other problems. Therefore the world's scientist thoughts have been diverted towards natural or comparatively safer ideas of using chemicals in agriculture. There is a growing interest about the use of phytochemicals for human's welfare. Phytochemicals are considered an important potential source for alternative agrochemicals. However, the ill effects of the plant derived compounds are least studies. This paper presents the field observations and review of the work done to elaborate the use of plant derived compounds for food production. Phytochemicals have long been recognized as a chemical warfare among the plant species. The intensive and repeated application of pesticides is effective for a specific pest but often results in several negative effects, such as the evolution of resistant weeds, residual effects on the following crops, and the disappearance of some susceptible pests, which affects overall biodiversity and environmental contamination. This deteriorating situation suggests that an environment-friendly agriculture is required to reduce the solely dependence on chemical herbicides for weed control. Exploitation of phytochemicals is a natural and environment-friendly technique having potential to be a unique tool for pest management and sustainable agriculture. Phytochemicals can also persist in soil, affecting the soil biota. Phytochemicals and agro-chemicals were applied as sole and in combination under field conditions. Statistically significant effect of phytochemicals on the soil micro-organisms was observed. This change in the soil fauna even at small scale can change the major functions of the agro-ecosystem at large scale. While review of the papers published in the last 10 years showed that the role of the phytochemicals is positive and negative as well. All types of chemicals (phytochemicals and agro-chemicals) affect the diversity of soil micro-organisms. However, the beneficial effects of the phytochemicals on the overall role in an agro-ecosystem is appreciable. Thus the nature provides un-limited opportunities to solve the problems of today.

**EFFICACY OF PESTICIDES FOR THE CONTROL OF HELIOTHES (*HELICOVERPA ARMIGERA* L.) OF CHICKPEA CROP UNDER THE AGRO EDAPHIC CONDITIONS OF THAL ZONE**

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Thal zone is characterized by sand dunes, where topography is erratic and uneven. Fields are cultivated on monsoon rainfalls. Chickpea is the most cultivated leguminous crop of this zone. Heliothes is the most obnoxious insect pest of this zone. As the crop boosts, *Heliothes* attack on the pods of the plants. Then it quickly pupates in the sandy loam soil to complete its hibernation

period. It becomes difficult to manage it. For this purpose, a field experiment was conducted at the farm of Mr. Muhammad Jamil, Dera Faiz Muhammad wala, Tehsil Kaloor Kot, District Bhakkar to test different insecticides available in the market against *Heliothes* to manage it. For this purpose different insecticides were tested against the *Heliothes* along with control. They were: T<sub>1</sub> Lannate 40 SP (Meyhomyl) @ 300 g/acre, T<sub>2</sub> Tracer 240 SC (Spinosad) 60 ml/acre, T<sub>3</sub> Steward 150 SC (Indoxacarb) @ 150 ml/acre, T<sub>5</sub> Curacuran 500 EC (Profenofos) @ 500 ml/acre, T<sub>6</sub> Reesham 1.9SC (Emmamectin) @ 100 ml/acre and T<sub>7</sub>Lorsban 40 EC (Chloropyrifos) @ 500 ml/acre. It was found that all the insecticides successfully controlled the pod borer infestation in chickpea as compared to control. Among all the insecticides, Reesham 1.9 SC (Emmamectin) @ 100 ml/acre proved to be very effective against the pod borer in chickpea as compared to rest of all other insecticides as the biomass produced was the highest in the treatment of this treatment. It is therefore suggested that Reesham 1.9 SC (Emmamectin) @ 100 ml/acre should be used for the better management of *Heliothes* in chickpea under the agro edaphic conditions of Thal zone.

#### STUDIES ON POPULATION ABUNDANCE OF MELON FRUIT FLY *BACTROCERA CUCURBITAE* (COQUILLET) IN VEGETABLES AGRO-ECOSYSTEM IN DISTRICT HYDERABAD

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The Melon Fruit Fly *Bactrocera cucurbitae* (Coq.) belongs to family; Tephritidae, order; Diptera and is distributed throughout the vegetable growing areas of Sindh, Pakistan. The *B. cucurbitae* is injurious pest of more than 125 species of the vegetables throughout the world. In the present studies we investigated the population of this important pest in cucurbit crops and influence of abiotic parameters such as; temperature, relative humidity and rainfall. The study was carried out at two different locations of District, Hyderabad. The locations were Jeay Shah and Dehli farm where three cucurbit vegetable crops, such as bottle gourd (*Lagenaria siceraria*), bitter melon (*Momordica charantia*) and ridge gourd (*Luffa acutangula*) were grown. The traps were baited with Cue-lure and deployed at three meter height in the all locations from 01.01.2015 and up to 30.06.2015. Results revealed that overall significantly higher ( $P < 0.05$ ) population was recorded on *L. acutangula*, *M. charantia* and *L. siceraria* (130.64, 127.21 and 122.91), respectively. However, significantly higher ( $P < 0.05$ ) population was observed on *L. acutangula* ( $339.4 \pm 22.59$ ) during the 4<sup>th</sup> week of May 2015 followed by *M. charantia* ( $334.6 \pm 22.76$ ) and *L. siceraria* ( $333.2 \pm 20.13$ ). Whereas; lowest population was recorded on *L. siceraria* ( $5.8 \pm 1.39$ ) followed by *L. acutangula* and *M. charantia* ( $6.8 \pm 0.80$ ,  $8.0 \pm 1.30$ ) respectively during the 4<sup>th</sup> week of January. The population of *B. cucurbitae* was significantly correlated with the temperature while negatively correlated with relative humidity. Results of the present investigation would be useful in developing a sustainable pest management strategy in the vegetables agro-ecosystem.

**SUSCEPTIBILITY OF STORED FRUIT OF DATE-PALM VARIETIES OF SINDH TO THE INFESTATION BY COLEOPTERAN PEST IN RELATION TO THEIR CHEMICAL COMPOSITION**

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The date palm fruit is a very important source of nutrients and cash crop of Sindh, Pakistan. The climatic conditions of Sindh are ideal for cultivation of date palm. There are many varieties of date palm cultivated in Sindh but commercially important varieties are Kupro, Karbalian, Aseel, Fasly and Dadhi. During storage process these varieties are infested by Sawtooth grain beetle *Oryzaephilus surinamensis* (L.) which cause severe damage to these stored varieties consequently these could not export to advanced countries. During present study the infestation and feeding behaviour of Sawtooth grain beetle *Oryzaephilus surinamensis* (L.) was observed on Kupro, Karbalian, Aseel, Fasly (semi-dry dates) and Aseel and Dadhi (dry dates) in relation to sugar, moisture and ash contents. The whole study carried out in laboratory from August 2011 to April 2013. The maximum mean infestation of Sawtooth grain beetle was recorded 40.42 Mean percentage on Kupro while the minimum mean infestation was recorded 22.85 on Aseel and Dadhi (dry dates). The highest sugar content and moisture percentage observed in Kupro 85.90% and 23%. Whereas lowest percentage was 65.50% and 10% in Aseel and Dadhi dry varieties. The mean infestation is positively correlated with sugar and moisture content. The maximum survival rate of Sawtooth grain beetle was recorded 88% on Kupro and lowest survival rate 46.51% on Dadhi variety of date palm. Present study revealed that Kupro and Karbalian semi-dry varieties are more attractive to Sawtooth grain beetle as compared to Aseel and Dadhi dry varieties. The reason of attraction was high sugar and moisture contents.

**OCCURRENCE OF SOIL MACRO-FAUNA AMONG OKRA (*ABELMOSCHUS ESCULENTUS* L.), TOMATO (*SOLANUM LYCOPERSICUM* L.) AND CAULIFLOWER (*BRASSICA OLERACEA*) FIELDS**

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The present study was conducted to record the occurrence of soil macro fauna among Okra (*Abelmoschus esculentus* L.), Tomato (*Solanum lycopersicum* L.) and Cauliflower (*Brassica oleracea*) fields" under the ecological conditions of district Faisalabad. From the whole results, it was recorded that among okra fields, total 469 specimens pertaining to 21 families, 24 genera and 30 species were collected. Maximum relative abundance 63.11% ( $n \geq 296$ ) was recorded for *Pheretima posthuma* (family Megascholoidae). Among cauliflower fields, total 499 specimens were collected and identified pertaining to 23 families, 42 genera and 50 species. Order Haplotaenidia, Pulmonata and Isoptera were among the prominent orders and maximum relative abundance 10.62% ( $n \geq 53$ ) was recorded for *Oniscus asellus* (family Oniscidae). From tomato

fields, maximum relative abundance 13.91% ( $n \geq 64$ ) was recorded for *Dolichonderus* spp. (family Formicidae). Peaks boots up were recorded along the vegetative stage and attain maximum frequency till ripening stage and then decreased. While, in case of cauliflower, such trend was not recorded and distribution was recorded in irregular trend for the occurrence of soil macro-fauna. From Okra fields highest relative abundance 63.11% ( $n = 296$ ) was recorded for genus *Pheritima* (family Megascholoidae); from cauliflower fields, maximum relative abundance 10.62% ( $n=53$ ) was noticed for the genus *Oniscus* (family Oniscidae) and from tomato fields, maximum relative abundance 21.30% ( $n=98$ ) was noticed for genus *Dolichonderus* (family Formicidae). For Okra fields, diversity was recorded as (0.0021); from cauliflower fields (0.0037) and from tomato fields (0.0052). The results of ANOVA between depending variables (Okra, Cauliflower and Tomato) and independent variables (soil macro-fauna) was recorded non significant ( $F = 0.01$ ;  $P = 0.9904$ ).

#### FACTORS AFFECTING THE NUMERICAL RESPONSE OF *COCCINELLA SEPTEMPUNCTATA* (L.)

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The numerical response of *Coccinella septempunctata* was studied in the laboratory against various prey groups at different temperature levels. The rate of egg laying was highly correlated with prey consumption ( $r^2 = 0.9521$   $P < 0.001$ ) as more prey consumed more energy the predator allocated for reproduction but within a certain range of temperature between 25-30°C. There was no ephemerons time of synchrony in emergence for both predator and prey in real field conditions. The prey comes earlier in the spring after long hibernation at very low temperature ranging from 6-10°C while the predators starts emerging at temperature 15°C. The minimum threshold temperature recorded for the normal activity of prey *Myzus persicae* was 8+1°C while the predator *C. septempunctata* was started appearing at 15+1°C. Similarly the minimum thresholds of diet requirement for the egg production was 3.5 aphids/arena/24 hours. However the oviposition behaviour of this predator was changed with changes of its surrounding. The predators response to the prey *M. persicae* in the experimental arena was ( $F=10.49P < 0.005$ ) when the female exposed to sharing partner ( $F = 6.34 P < 0.005$ ). Although this predator was considered as general feeder but in present study the factor of prey preference was observed as female lay more eggs when fed on *Myzus persicae*. There was a significant difference in the rate of egg production when predator fed on *Myzus persicae* compared those of *Rhopalosiphum padi* and *Brassicorhynchus brassicae*.

#### SCREENING FOR BRUCHIDS (*CALLOSOBRUCHUS MACULATUS*) RESISTANCE IN MUNGBEAN GENOTYPES UNDER LAB CONDITION

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. Pulse beetles (*Callosobruchus maculatus*) (F.) are the principal post-harvest pest of mungbean and other stored pulses. In storage, the adult female lay eggs directly on seed coat. The newly hatched larva bore through the egg shell and penetrates seed coat, continue to feed and

complete their development inside the seed. After completion, the insects emerge as adult beetles leaving behind a hole at the exit point. Bruchids infestation causes reductions in the weight, seed viability, sale ability and infested grains unfit for human consumption. The alternative to chemicals and other control measures is to develop bruchids resistant genotypes required for storage purposes. The entomological research on bruchids is aimed at screening progenies obtained from crosses made by Mungbean Breeder for resistance/tolerance to beetles. Culture of bruchids beetle was maintained on bold mungbean grains at  $28 \pm 2^{\circ}\text{C}$  and  $70 \pm 5\%$  relative humidity. Insect of uniform age males and females were collected separately by isolating mungbean grains in small transparent glass test tubes mouth plugged with cotton. 10-15 pairs of newly emerged adults were collected within 24 hours and released in glass/plastic jars containing sound grains of mungbean. The jars were covered with muslin cloth to facilitate aeration. The stock culture maintained was utilized for conducting the experiment. 28 mungbean crossed genotypes were evaluated to ascertain their resistance to bruchid beetles. The resistance of mungbean genotypes to bruchids was evaluated on the basis of % grain damage, % grain infestation and adult emergence. The results indicated that none of the tested genotypes was completely resistant to bruchid attack. Out of tested genotypes screened for resistance to bruchids (*Callosobruchus maculatus* F.) along with the seeds of six parents (V1128, V2709, V2802, V2817, Ramazan and NM 2006, only a genotype V1128 having 3% grain damaged showed tolerance to bruchids in current screening test. All the evaluated recombinants were found susceptible.

**ECOLOGICAL SIGNIFICANCE, INFESTATION LEVEL AND CONVENTIONAL CONTROL AGAINST *AOELESTIS SARTA* SOLSKY (COLEOPTERA: CERAMBYCIDAE) IN DISTRICT QUETTA, BALUCHISTAN**

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*Aoeslestis sarta* (Quetta borer) Solsky is ecologically the most important pest causing severe damage to wood by declining both the quality and quantity. Studies were carried out in field during 2013-2014 in localities of District Quetta selected for this study. Total 350 tree varieties (Pinus, Safeda (Himalayan poplar), Santroza, Apricot, Golden delicious, Red delicious and Amri varieties of apple) were studied, and level of infestation was evaluated by counting the number of life holes made by the first instar larvae. Out of 350 trees, 192 (62.79%) were found badly infested by *A. sarta*. Among all the trees, apple varieties (golden and red delicious) were observed the most infested hosts with 87.28% and 72.60% respectively. Mean infestation was found higher in other limbs comparatively than main trunk. Ecological significance of apple tree groups (Golden, Red delicious, and Amri varieties) were examined in terms of cost reduction taken by healthy, infested and dead wood price per Kg in market. 70-80% market lost of wood was observed. Level of infestation was recorded on 23 years old trees and conformed that aged group varieties were more infested than young ones. Relationship between the life holes, diameter and height of the tree were established and concluded that old aged and high width bearing tree were more infested and contained large number of life holes. Control strategies applied were conventional seasonal pruning and insect repellent technique. Seasonal pruning was further grouped as spring and autumn pruning. Each season divided into three groups including one control. The results of autumn season pruning showed efficient control of first larval instar by decreasing the infestation rate 32.3%-35.71% than spring season 9.87%-10.85%. Insect repellent technique was used before *S. sarta* adult emergence, and selected sites was classified into three groups including one control (untreated).

Insect repellent technique was found completely ineffective with an increase in mean life holes made by the larvae of the pest and to seize the activity of adult *S. sarta* females to lay eggs.

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**IMPACT OF SOME NON-REPELLENT INSECTICIDES AND IGRS ON THE  
TUNNELING AND TRAIL FOLLOWING BEHAVIOUR OF SUBTERRANEAN  
TERMITE, HETEROTERMES INDICOLA (WASMANN) (ISOPTERA:  
RHINOTERMITIDAE)**

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The subterranean termite *Heterotermes indicola* (Wasmann) is one of the most economically important and destructive pest species in Pakistan. Laboratory studies were conducted to evaluate the impact of various concentrations of non-repellent insecticides and insect growth regulators i.e. fipronil, indoxacarb, chlorfenapyr, imidacloprid, hexaflumuron and lufenuron respectively on tunneling and trail following behaviour of *H. indicola*. It was observed that sand soaked with  $\geq 1$  ppm of fipronil restricted tunneling and could create effective barrier against termite workers. Fipronil at  $\leq 0.5$  ppm did not affect the trail following ability and 70 - 90% termites successfully completed the trail. Tunneling observed to be greatly reduced in  $\geq 5$  ppm of indoxacarb treated sand but mortality remained  $< 50\%$  whereas in 50 ppm treated sand 100% mortality was recorded. Indoxacarb at  $\leq 20$  ppm did not affect trail following. In contrast with indoxacarb termites tunneled freely in chlorfenapyr treated sand and maximum mortality recorded was 91.6% in 1 ppm treated sand showing chlorfenapyr could be good candidate for soil treatment. Chlorfenapyr did not showed significant impact on trail following of termites that were exposed  $\leq 3$  ppm treated sand. Imidacloprid acted more like repellent insecticide and allowed little tunneling in treated sand and seriously hampered trail following ability. Termites treated with 50 – 100 ppm imidacloprid completely failed to follow the trail. Tunneling was greatly reduced in sand treated with  $\geq 500$  ppm of hexaflumuron whereas trail following ability and walking speed was not affected significantly at all the tested concentrations. Similarly tunneling was greatly reduced in sand treated with  $> 1000$  ppm of lufenuron but trail following ability was not affected at any tested concentration of lufenuron.

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**BIOLOGICAL CONTROL OF MOSQUITO LARVAE USING EDIBLE FISH**

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Mosquito control has been a point of focus in scientific communities and health-providing units. Various methods have been tried and still under-way, to eliminate mosquito population. However, certain environmental issues related with chemical control of mosquitoes have convinced experts to opt for eco-friendly methods. Present study was focused on using edible fish as predator of mosquito larvae. Experimental results revealed that, edible fish have tremendous potential to be used as larvivorous predator of mosquito. It was further found that, larvivorous fish (*Gambusia*



*Affinis*), used in this study preferred live larvae when compared with commercial food. The rate of consumption was between 180 to 190 larvae per fish per day, and fish preferred live larvae more than dead larval stuff. The percentage of larvae eaten from dead stock was 37.14% as compared to 65.72% from live larval stock. Another aim was to determine effect of light and dark on larvae consumption and it revealed that, 88.75% of larvae were eaten in the presence of light and 51.25% in dark. In micro field condition out of 500 larvae, only 13 (2.6 %) larvae were left, whereas rests of the larvae were consumed by the predator fish. Present finding reveal that, larvivorous fish tried as biological control agent against mosquitoes.

**STUDY OF INSECT PEST OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL)  
BELONGS TO FAMILY *SOLANACEAE* AND THEIR HOST PLANT SELECTION**

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Tomato (*Lycopersicon esculentum* L.), is one of the most nutritious vegetable of the world. It belongs to family *Solanaceae* and considered as significant vegetable in Pakistan. Baluchistan contribute to 43% of the total tomato production and is ranked as second most important vegetable crop after potato and is a rich source of vitamins A, B, C, iron and Phosphorous. Grower often apply pesticides in order to protect their investment, this leads to development of insect resistance, environmental contamination and poor management of pests. Host plant resistance offers a viable alternative to the use of chemical insecticides for managing insects infesting tomato. Owing to limited availability of improved cultivars, hence the identification of improved tomato varieties, high yielding and insect resistant are necessary, therefore an experiment will be conducted in Baluchistan Agricultural Research and Development Centre (BARDC) Quetta, Baluchistan, during the year 2014-15 to evaluate varieties for high resistant. The relative abundance and diversity of insect species will be studied on different varieties of tomato (*Solanum lycopersicon* L.) by using RCBD with three replication of single variety under field conditions. Higher and lower abundance of insect pest on different tomato plant variety will be recorded at regular interval till at the maturity of the plant. The insect pest will be identified from the insect museum NARC. Collected data of insect pest will be recorded and will be subjected to statistical analysis. Based on further laboratory studies on the various mechanisms and bases of resistance, host plant resistance is planned to utilize the physico-morphic characters of tomato cultivars for safe control and environmental friendly. The use of resistant cultivars in agriculture is a safe and environmental friendly technique to avoid the use of insecticides. In preliminary screening the physical and chemical characters of tomato cultivars will be used in the experimentation. After the identification and selection of different physical characters of plants will be used for comparatively resistance and susceptibility level of tomato cultivars against different insect pest. The various Physico-morphic characteristics and chemical analysis will be help to known the resistance of the tomato cultivars. Finding of my research will be helpful in finding the resistant host-plant selection and population dynamics of insect pests of tomato will helpful for the proper management and high yield.

**EFFECTS OF CYPERMETHRIN ON BIOLOGY OF MELON FRUIT FLY *BACTROCERA CUCURBITAE***

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Toxic effects of cypermethrin were studied on melon fruit fly, *Bactrocera cucurbitae* (Coq.). Third instar larvae were treated with different doses of cypermethrin i.e. 0.0016%, 0.0032%, 0.0063%, 0.0125% and 0.025% by contact method. LD<sub>50</sub> against larvae of melon fruit fly was found to be 0.00647%. Treated insects were left for 24 hours for the evaluation of toxic effects and for the determination of lethal dose concentration. Surviving insects were kept for the observation of percent pupal formation, emergence of adults, egg laying (fecundity) and hatching (fertility). The lowest concentration of cypermethrin i.e. 0.000245% showed 1% mortality, while 0.0354% concentration of cypermethrin caused 90% mortality. As a result of a high dose 0.025% of cypermethrin against 50 larvae, only 9.66 larvae were succeeded to pupate among them only three pupae succeeded to emerge as adult. Similarly, the insects treated with the same dose i.e 0.025% showed 69.95% fecundity and 30.05% fecundity inhibition while 75.67% fertility and 24.33% fertility inhibition. Fecundity inhibition was not reciprocal to ascending concentration of cypermethrin.

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## SECTION – I I I

## ENTOMOLOGY

## THE POPULATION DYNAMICS OF MAJOR CHEWING INSECTS PESTS OF SUGARCANE IN JHANG, PUNJAB, PAKISTAN

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The experiment was carried out for making an overall view population dynamics of major chewing insects/ pests complex of sugarcane in Distt. Jhang, Punjab, Pakistan. The surveillance of top borer (*Scirpophaga nivella*), root borer (*Emmalocera depressella*) and stem borer (*Chilo infuscatellus*) were observed in spring sown sugarcane crop during the year 2011. From the results, it was concluded that the populations of top borer (*Scirpophaga nivella*), root borer (*Emmalocera depressella*) and stem borer (*Chilo infuscatellus*) were at maximum appearance in month of May, April-May and May, respectively, on surveyed sugarcane crop. The correlation of sugarcane top and stem borer populations were observed as positively with temperature and negatively with relative humidity.

THE FITNESS COMPARISON AMONG SUSCEPTIBLE AND SELECTED RESISTANT STRAINS OF *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE)

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The life history parameters of three resistant strains (M<sub>1</sub>, M<sub>2</sub> and SWL) and the susceptible strain of *Bactrocera zonata* were compared to evaluate comparison fitness among these strains. Larval duration, pupal duration, development times and generation times of susceptible strain was lower as compared to that of three resistant strains. The fecundity, emergence rate, net replacement, adult male and female longevity were found higher in the susceptible strain to that of other three resistant strain. It was concluded that all the resistant strains complete their life cycle in prolonged period as compared to susceptible one. Intrinsic rate of increase and biotic potential were observed high in the susceptible strain (0.18 and 0.66) as compared to other three resistant strains.

Relative biotic potential and relative fitness were remained maximum in M<sub>2</sub> resistant strain (0.88 and 0.70), when compared to M<sub>1</sub> and SWL resistant strains.

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#### PREVALENCE OF DESERT LOCUST (ORTHOPTERA) FROM VARIOUS LOCALITIES OF THAR REGION SINDH

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A study of various localities of Thar Desert was carried out from January 2015 to October 2015. In this survey 4 species of locusts; belonging to 4 genera were reported from Thar Desert. Collected data showed that occurrence of *Schistocerca gregaria* was very high with 283 No. and 74.67% followed by *Anacridium aegyptium* with 58 No. and 15.3%, *Cyrtacanthacris tatarica* with 21 No. and 5.54% and *Locusta migratoria* with 17 No. and 4.48%. Incidence of desert locust was increased after monsoon rains in the month of August, when condition became favorable since first monsoon rains their number increased in following months i.e. September and October. Currently in the last days of October and start of November desert locust is expanding with dramatic ratio in areas confined to a locality of Mahandre-Jo-Par. *A. aegyptium* and *C. tatarica* were reported for first time from this region, *A. aegyptium* were present all the time in study area and *C. tatarica* were appeared after monsoon rains. These four species of locusts are destructive to crops and other vegetation, swarms of desert locust can damage vegetation on large scale. These are initial findings further study is in progress its exact status will be cleared later on.

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#### STUDY ON THE FINDING OF AVIAN PREDATION ON CO-EXISTING POPULATION OF INSECT

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At present 05 localities from Tandojam were surveyed for analysing the birds and insect population. Over all 526 samples of birds were observed along with 844 samples of insects. The prevalence of birds species along with the estimated population showed that 03 birds group i.e. crow, warblers, common Myna and Babblers were found frequently than other birds species, some of the such as Rosy pastor, jungle, sparrow, yellow-throated sparrows were found less in frequency but high numbers i.e. large flocks observed but on few occasions. Insect was found maximum in family Acrididae i.e. *Hieroglyphus perpolita*, *H. Oryzivorus*, *H. nigrorepletus* from sub family Hemiacridinae, *Locusta migratoria* from sub family oedipodinae and *Schistocerca gregaria* from sub family cyrtacanthacridinae. A part from the locust and grasshoppers Aphid, painted bug and mustard saw fly were also preferred by birds. This is the first attempt of such comparative examination of co-existing population of birds and insects in this permissive.

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**SEASONAL MONITORING OF FRUIT FLY *BACTROCERA ZONATA* THROUGH  
INSTALLATION OF METHYL EUGENOL TRAPS IN GUAVA ORCHARD LARKANA,  
SINDH**

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Fruit fly is a serious threat to production and quality of fruits particularly mango and guava in relation to Sindh province. Male adult population of fruit fly species captured through methyl eugenol pheromone traps installed at surface, 1 metre, 2 metres and 3 metres height were maximum at Mahoota Guava Farm, Larkana with  $2116.74 \pm 18.91$ ,  $1973.32 \pm 17.33$ ,  $2208.39 \pm 20.58$  and  $1967.77 \pm 17.90$  trap catches. *B. dorsalis* catches in methyl eugenol pheromone traps installed at surface, 1 metre, 2 metres and 3 metres height were also highest at Mahoota Guava Farm, Larkana with  $25.46 \pm 0.20$ ,  $24.22 \pm 0.20$ ,  $26.57 \pm 0.21$  and  $23.94 \pm 0.20$  trap catches. Regardless the trap height, *B. zonata* and *B. dorsalis* infestation was highest when the methyl eugenol pheromone traps were installed at 2 metre height; while traps installed at 1 metre and 3 metre height could not catch *B. zonata* and *B. dorsalis* more than surface installed traps.

**DISTRIBUTION PATTERN OF VARIOUS SPECIES OF TETTIGONIOIDEA (   
ENSIFERA) OF PAKISTAN**

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Pakistan is a biogeographically diverse region having rich vegetation that offer ideal breeding places to varieties of insects including Tettigoniodea (commonly known as katydids). During present study fair large No. of Tettigoniodea were captured from different ecological zones of Pakistan. The collected material comprise on 07 sub-families *i.e.*, Pseudophyllinae, Phaneropterinae, Conocephalinae, Tettigoniinae, Hexacentrinae, Mecopodinae and Decticinae belonging to various species with the greatest economic importance. Phaneropterinae and Conocephalinae showed its wide diversity throughout Pakistan while Tettigoniinae, Pseudophyllinae and Mecopodinae were seen rare in collection the possible reason of this discrepancy might be feeding on nutritional rich vegetations and favourable climatic condition of region and non-availability of these resources. Nevertheless, a brief description of each supra-generic category of Tettigoniodea along with photographs and synonymy is also documented. Additionally, detailed list of host plants from Pakistan was also composed for the first time. It was noticed that katydids having a close association with *Citrillus colycynthis*, *Dactyloctenium scindicum* and *Poa tenella* in the survey areas. These findings supplied important basis and data for integrated Pest Management (IPM) of Tettigoniodea biodiversity conservation and grassland restoration in Pakistan. This research project No.PSF/Res/S-SU/Bio (423) is financially supported by Pakistan Science Foundation Islamabad.

**DISTRIBUTION OF THE GENUS *SPHINGONOTUS* FIEBER (OEDIPODINAE:  
ACRIDIDAE: ACRIDOIDEA : ORTHOPTERA) FROM PAKISTAN**

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As a result of present work 12 species and subspecies of genus *Sphingonotus* were reported from Pakistan that include: *Sphingonotus savignyi* Saussure, *S. hussaini* Baloch & Wagan, *S. longipennis* Saussure, *S. nebulosis tokhai*, *S. balteatus himalayanus* Uvarov, *S. balteatus balucha* Uvarov, *S. sindhensis* S. akbari Wagan & Baloch, *S. maculatus spetraeus* Bei-Bienko, *S. rubescens afghanicus* Mistchenko, *S. rubescens subfasciatus* Mistchenko, and *S. rubescens rubescens* (Walker). Beside the study on the distribution of this genus a detail observation was also provided along with illustration on phallic complex for more authentic identification of closely related species of this group.

**DIVERSITY OF PYRGOMORPHIDAE (PYRGOMORPHIDOIDEA: ORTHOPTERA)  
FROM PAKISTAN**

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Pyrgomorphidae is considered as the major potential pest of many ornamental plants, vegetables and other crops, but mostly found in rice, cotton, sugar cane and pineapple plantation. During the present survey a total of 2100 specimens were collected from different ecological zones of four provinces of Pakistan during the year 2013-2015 in various months of the year. The collected material was sorted out into 04 tribes' i-e Chrotogonini, Pyrgomorphini, Atractomorphini and Poekilocerini, belonging to 05 genera viz; *Chrotogonus*, *Tenuitarus*, *Pyrgomorpha*, and *Poekilocerus*. Beside this 10 species *Chrotogonus trachypterus trachypterus* (Blanchard, 1836), *C. trachypterus robertsi* Kirby 1905, *C. turanicus* Kuthy 1905, *Tenuitarus orientalis* Kevan 1959, *T. angustus* (Blanchard, 1836), *Pyrgomorpha bispinosa bispinosa* Walker 1870, *P. bispinosa deserti* Beinko & Mischenko 1951, *P. hemiptera* Uvarov 1938, *Atractomorpha acutipennis blanchardi* Bolivar 1905, *Poekilocerus pictus* (Fabricius 1775) was also re-described.

**STUDY ON THE DEVELOPMENTAL STAGES OF THREE OEDIPODINAE SPECIES  
(ORTHOPTERA) FROM SINDH**

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Oedipodinae is among widely distributed voracious feeder of many economically important

crops such as sugarcane, wheat, millet, maize, fodder crops, as well as wild vegetations of Sindh. Presently developmental stages of three dominant pest species i-e *Aiolopus thalassinus thalassinus* (Fabricius), *Acrotylus humbertinus* (Saussure) and *Trilophidia annulata* (Thunberg) were studied under laboratory condition to confirm exact number of molts and nymphal stages of species. It was found that first instar hatch from vermiform larva. Usually there are five molt and six nymphal stages and adult being the seventh one. Hoppers appear during June to October while in case of *A. thalassinus thalassinus* hopper were found throughout the year with lesser and greater ratio on different habitats (with exception in January). Morphometric analysis on the developmental stages of all three studied species highlighted significant differences in various body parts, morphology and coloration pattern was also found diverse. Beside this, brief comparative description, illustration identification keys were also constructed for the separation of various developmental stages. Addition to this, feeding behavior and incidence of hoppers and adults were also observed in field.

#### BIODIVERSITY OF CALIFERANS SPECIES (ORTHOPTERA) FROM THAR DESERT

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Thar Desert is considered as the seventh largest desert on planet Earth and third in continent Asia. It covers large area of Pakistan and India which is about 175,000 square kilometers covering districts of Tharparkar, Mirpurkhas, Umerkot, Khairpur, Sukkur and Ghotki in Sindh province and without doubt the most inhospitable eco-region in the Indo-Pacific region. In order to know its hidden wealth of califera, an attempt was made to collect the large number of specimens from different localities of Thar Desert during year 2014-2015. In the result of extensive survey about 523 specimens were captured and sorted out into 3-families Viz: Acrididae, Dericorythidae and Pyrgomorphidae along with 14 species 08 were identified i.e. *Heteracris littoralis* (Rambur, 1838), *Dericorys tibialis* Pallas, 1773), *Pyrgomorpha* (*Pyrgomorpha*) *bispinosa bispinosa* Walker, 1870, *Chrotogonus* (*Chrotogonus*) *homalodemus homalodemus* (Blanchard, 1836), *Tropidopola graeca graeca* Uvarov, 1926, *Mesopsis cylindricus* (Kirby, 1914), *Acrida exaltata* (Walker, 1859), *Sphingonotus* (*Sphingonotus*) *rubescens rubescens* (Walker, 1870) while other are in process of identification.

#### PRELIMINARY STUDY ON THE PRAYING MANTIDS (DICTYOPTERA: MANTODEA) FROM DISTRICT SANGHAR

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Mantids are ambush 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 survey was carried out in various localities of Sanghar and about 48 specimens have been collected and identified as: *Empusa unicornis* (Saussure, 1871), *Blepharopsis mendica* (Fabricius, 1775), *Humbertiella indica* (Saussure, 1869), *Iris splendida* (Uvarov, 1922),

*Iris oratoria* (Linne, 1758) while *Deiphobe infusata* (Saussure) have been reported for the first time from this region. Hopefully, more extensive survey will enhance the further wealth of mantids fauna from this region.

#### COMPARATIVE STUDY ON THE DIVERSITY OF PHANEROPTERINAE (TETTIGONIOIDEA: ORTHOPTERA) FROM PAKISTAN

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Phaneropterinae are phytophagous insects some of the species are important pests of agricultural crops while many species are ecologically associated with forest biocenoses, damaging trees and shrubs. During the present investigation 21 species pertaining to 08 tribes and 08 genera has been recorded. Out of which 17 species i.e., *Trigonocorypha unicolor*, Stål, 1873, *Trigonocorypha angustata*, Uvarov, 1922, *Trigonocorypha nr. angustata* Uvarov, 1922, *Phaneroptera bivittata* Bei-Bienko, 1954, *Holochlora nigrotympana* Ingrisch, 1990, *Holochlora venosa* Stål, 1873, *Holochlora astylata* Karny, 1926, *Ducetia japonica*, Thunberg, 1815, *Letana rufonotata* (Serville, 1838), *Letana bulbosa* Ingrisch, 1990, *Tylopsis lilifolia* Fabricius, 1793, *Isopsera spinosa* Ingrisch, 1990, *Isopsera stylata* Brunner von Wattenwyl, 1878, *Isopsera pedunculata* Brunner von Wattenwyl, 1878, *Isopsera astylata* Karny, 1926, *Himertula kinneari* (Uvarov, 1923) and *Himertula marmorata* (Brunner von Wattenwyl, 1891) constructed new regional record for Pakistan. While 04 species i.e.; *Phaneroptera spinosa*, Bei-Bienko, 1965, *Phaneroptera roseata*, Walker, 1869, *Phaneroptera gracilis* Bei-Bienko, 1954 and *Holochlora japonica*, Brunner von Wattenwyl, 1878 were re-described. Moreover, the record collection of Phaneroptera represents the first comprehensive record of the entomological fauna of Pakistan. Financial support received from Pakistan Science Foundation Islamabad (PSF) for Research Project No.PSF/Res/S-SU/Bio (423) and Higher Education Commission Islamabad (HEC) under Indigenous Ph.D Fellowship.

#### STUDY ON THE DIFFERENTIATION AND DISTRIBUTION OF IMMATURE SLANT-FACED GRASSHOPPER (ACRIDINAE: ACRIDIDAE: ORTHOPTERA)

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The slant-faced grasshoppers belonging to Sub-family Gomphocerinae are of considerable economic importance to rangeland and agricultural crops. During the present immature of 04-species namely *Oxypterna afghana* Ramme, 1952, *Ochrilidia geniculata* (Bolívar, 1913), *O. gracilis gracilis* (Krauss, 1902), *Aulacobothrus lutipes lutipes* (Walker, 1871) were studied. There were significant differences were found in structure, color and color patterns of all immature stages. Further, hatching of immature was also found different in various climatic conditions. Key,



description and illustration was also provided for easily identification of instars. All these 04-species passed through five nymphal instars before becoming adult. Hoppers mostly feed on *Cymbopogon jwarancusa* and *Cynodon dactylon*.

### **BIOLOGY OF OXYA SPECIES (OXYINAE: ACRIDIDAE: ORTHOPTERA) UNDER LABORATORY CONDITIONS**

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The rice plant is vulnerable to many insects from its sowing to harvest. Amongst the pests *Oxya* is considered its major pest and destroyed its all developmental stages i.e. seeding, tailoring and stem elongation. In order to know its hatching and other life parameters of 04 *Oxya* species were reared under laboratory conditions and following data was analyzed. The observation regarding biological parameter highlighted that age of maturation of insect was  $5.0 \pm 0.79$  days for male and  $0.90 \pm 0.41$  days for female in *Oxya hyla hyla* while it was  $5.50 \pm 0.9$  days for male and  $7.00 \pm 0.50$  days for female in *Oxya velox*. Similarly duration of pre-copulatory period was noted  $3.0 \pm 0.79$  days in *O. hyla hyla* and  $3.30 \pm 0.75$  days in *O. velox*. Total mating was noted  $5.60 \pm 1.14$  for male  $8.20 \pm 1.48$  for female in *O. hyla hyla* and  $5.0 \pm 1.00$  for male and  $8.20 \pm 0.53$  for female in *O. velox*. Beside this, interval between each oviposition was note  $4.75 \pm 1.70$  days and  $5.00 \pm 1.00$  days in *O. hyla hyla* and *O. velox* respectively. However, duration of oviposition in *O. hyla hyla* was  $17.2 \pm 3.0$  mints and  $17.00 \pm 2.00$  mints were noted in *O. hyla hyla* and *O. velox* respectively. Secretion of foamy mass takes place  $5.50 \pm 1.29$  and  $6.33 \pm 1.52$  (mints) in *O. hyla hyla* and *O. velox* respectively. This work is first of its kind and will be of great help to institutions / agencies dealing with the pest management.

### **CONTRIBUTION OF ASPERGILLUS SPECIES IN BIOLOGICAL CONTROL TO REDUCE RICE PEST POPULATION**

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*Aspergillus* are an important entomopathogenic fungi playing vital role in regulation of different pest species. During present investigation 04 species of sub-family Oxyinae viz: *Oxya hyla hyla* Serville, 1831, *Oxyavelox* Fabricius, 1787, *Oxya fuscovittata* Marschall, 1836 and *Oxya bidentata* Willemse, 1925 and 01 i.e., *Spathosternum prasiniferum* (Walker) from Subfamily Spathosterninae were treated with 03 *Aspergillus* species i.e., *Aspergillus niger*, *A. flavus* and *A. fumigatus* under laboratory condition. The prepared medium was inserted on the pronotum sheath of grasshoppers after the insertion of entomopathogenic fungi insect die within 7th day. However, maximum morality was noticed by day 5<sup>th</sup>. Present study suggests that these bio-control agents would be proved very useful tool in declining pest population. These 02 subfamilies having great economic important as sever pest of rice the most valued and eatable crop in Sindh including World.

### ON THE FINDING OF GRYLLIDAE (ORTHOPTERA: INSECTA) FROM THAR DESERT

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Thar Desert having very peculiar characteristic regarding its habitat but very poor sampling is available due to extreme climatic condition. At the present, an attempt was made and two extensive surveys were carried out in different months of the year during 2015 from Nangarparkar, Mithi, Diplo, Chachro. Gryllidae for the sampling of Gryllidae that are severe pest of the agriculture crops i.e wheat, maize, cotton and grasses. A total of 157 specimens were come in collection and were sorted out into 03 genera such as *Acheta* Linnaeus, , *Gryllus*, Linnaeus, and *Gryllodes*, Walker belonging to 04 species i.e *Acheta domestica* (Linnaeus 1758), *Gryllus bimaculatus* (De-Geer, 1773), *Gryllodes sigillatus* (Walker , 1869) and, *Gryllodes supplicans* ( Walker, 1859). It was also reported that more dominant species was *G. sigillatus* compare to other studied species.

### EFFECT OF A BIOTIC STRESS ON FEEDING POTENTIAL OF SEVEN SPOTTED LADYBEETLE, *COCCINELLA SEPTUMPUNCTATA*

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The biotic stress of seven spotted lady bird beetle, *Coccinella septumpunctata* (Linn) after 24 hour and 48 hour starvation was studied under laboratory conditions on canola aphid (*Lipaphis erysimi*), wheat aphid (*Schizaphis graminum*) and cabbage aphid (*Brevicoryne brassicae*). The experiment was done in petri dishes, having three replications. The results revealed that there is significant difference between consumption of *L. erysimi* species by adult beetle after 24 and 48 hours biotic stress. After 24 hour biotic stress the feeding was 143.3 and after 48 hour biotic stress the result was 86.3 aphid consumption. Fourth and third instar larvae of *C. septumpunctata* consumed the highest number of aphids of *L. erysimi*, after 24 hours, 48 hours biotic stress, the consumption was 79.7 and 146.3 respectively. While maximum consumption of *L. erysimi* by 3<sup>rd</sup> instar after 48 hour starvation was 143.3. From the study it is clear that the *C. septumpunctata* showed high feeding performance on canola aphids *L. erysimi* as compared to other aphid species as; *S. graminum* and *B. brassicae*.

### DISTRIBUTION AND ABUNDANCE OF SCELIO (SCELIONIDAE: HYMENOPTERA) ON ACRIDID'S POPULATION

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The genus *Scelio* is considered as larger within the family Scelionidae commonly known as parasitic wasp usually target eggs of Acrididae species throughout the world and some species have

been implemented as biological control agents. During present survey it was noticed that most important factor influencing distribution and abundance of *Scelio* species is host availability which may be determined by endogenous factors operating cyclically. Furthermore, *Scelio* may display host conservation strategies, limiting their own abundance in times of host shortage. Beside this, abiotic factors, principally rainfall and temperature, mainly have an indirect influence on parasitoid abundance through their effect on the host.

**STUDY ON THE CONSUMPTION INDEX AND GROWTH RATE OF *ACROTYLUS HUMBERTIANUS* SAUSSURE ON DIFFERENT DIET THROUGH SCANNING ELECTRON MICROSCOPE (SEM)**

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*Acrotylus humbertianus* Saussure is a major agriculture pest in Sindh. This species consumes a wide variety of food plants from different economic important families. In order to test its preference on different diets a conformity chemical analysis has been done through Scanning Electron Microscope (SEM) for the first time. The maximum consumption index (CI) of all stages *A. humbertianus* on cabbage was recorded 0.042 - 0.78 mg/day followed by 0.2-0.42mg/d on sugarcane and 0.019-0.43mg/d on maize, while least CI was calculated i-e 0.016-0.39 mg/day on mix diet. Beside this, growth rate (GR) of *A. humbertianus* on these food plants indicates that (GR) was highest on sugarcane i-e 0.016-0.25mg/d followed by 0.02 -0.62mg/d and 0.013-0.35mg/d on cabbage and maize respectively, while it was 0.011-0.32 mg/d on mixed diet when subjected to one way ANOVA, a significant difference in the consumption index on all stages of *A. humbertianus* on four host plants was obtained. The first and second instars of *A. humbertianus* differed significantly in their consumption indices on all four host plants and the highest consumption index was recorded on adult female fed on cabbage (0.78), while the growth rate (GR) of *A. humbertianus* on four plants i-e sugarcane, cabbage, maize, mixed diet was observed highest on cabbage i-e 0.040mg/ g day followed by maize, sugarcane and mixed diet i-e 0.031 mg/g day, 0.029 mg/g day and 0.020 mg/g day respectively. From nymphal stages highest growth rate was recorded in second and third instars i-e 0.043 mg/g day, fed on cabbage.

**PREVALENCE OF GROUND BEETLES (CARABIDAE: COLEOPTERA) FROM DISTRICT MIRPUR KHAS SINDH**

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Ground beetles are a large, cosmopolitan family of beetles, Carabidae, with more than 40,000 species worldwide, around 2,000 of which are found in North America and 2,700 in Europe. It is one of the top-10 largest animal families, as of 2015. In present studies two species from family (Carabidae: Coleoptera) were collected from district Mirpur Khas. About 10 ♂, 6 ♀ specimens of

*Scarites quadricaps* (Fabricius: 1775) and 5 ♂, 10 ♀ specimens of *Calosoma chinense* (Kirby: 1819) were examined. The species were identified on dissecting binocular compound microscope by using authentic keys and related literature.

**BIOLOGY OF *AILOPUS THALASSINUS THALASSINUS* (ACRIDIDAE: OTHOPTERA) FROM DISTRICT DADU**

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The genus *Aiolopus thalassinus* represents one of the most remarkable member of the order orthoptera, *Aiolopus thalassinus* is reported as a major pest of rice, sugar cane, wheat, maize & alfa alfa 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 Dadu city, Phulji, village Makhdoom Bilawal, village Bhawalpur, Village Sita, and Village Maloja Panhwar during the year 2013-2015.

**ABUNDANCE AND DISRIBUTION OF SCARAB BEETLES (COLEOPTERA: SCARABAEIDAE) IN LARKANA, SINDH**

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Scarab beetles belong to order Coleoptera (largest order of Insecta) and family Scarabaeidae with approximately 30,000 species of beetles throughout the world. Adults of many scarab beetles are noticeable due to their relatively large size, bright colors, often elaborate ornamentation, and interesting life histories. Weekly observations were made and the specimens were collected by multiple methods (mercury light trap, pitfall trap and hand picking) result showed that the abundance is correlated with lunar cycle, temperature and physical factors.

**SOUND PRODUCING ORGANS AND SONG PATTERNS IN *ACHETA* SP. (ORTHOPTERA: GRILLIDAE) WITH SPECIAL REFERENCE TO ITS SYSTEMATIC DIFFERENCES FROM THOSE IN *A. DOMESTICUS* (L.) THE ONLY OTHER SPECIES KNOWN FROM PAKISTAN**

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In the present work probably a new species of *Acheta* was reported from Punjab, Pakistan and described on the basis of its sound producing organs i. e., Tegmen, structure and number of

teeth present on stridulatory file as well as the different patterns of male songs. Previously only *Acheta domesticus* was known from Pakistan. Present study approves the importance of male calling songs as taxonomic character of this group. The unique nature of stridulating sound pattern, dominant song frequency and sound producing organs considered as the most important characters for the identification of *Acheta sp.* The songs and sound producing organs of the presented species are compared here not only with that of *A. domesticus* but also with those species reported in the literature to date. Khalifa (1950) stated that *A. domesticus* from Asia became cosmopolitan. Ghouri (1961) discussed the origination of *Acheta* from Africa or South eastern Asia. Alexander (1957) redescribed the taxonomy of different species of *Acheta*. The male producing calling songs are stereotyped, species specific and relatively simple in their patterns (Alexander 1962, Walker 1962, Doherty 1985). Recently Ahmad and Khan (2015) redescribed *A. domesticus* from Pakistan on the basis of its tegmen with venation and male genitalia. Specimens of *Acheta sp.* were collected from Punjab, Pakistan, usually at night. Male songs were recorded with the help of Zoom H4 next recorder and the songs were sliced by using Audacity portable Digital Audio Editor Version 1.2.6. These sounds were analyzed and compared by the use of software Praat 5400\_win 32. After preservation, the specimens were boiled to soften for detaching the right tegmen, for photograph by using Nikon Cool Pix 5400 digital camera having placed it under Nikon SMZ 800 Binocular. Morphological study of the stridulatory organs were analyzed with the help of Scanning Electron Microscope (SEM) photographs were further analyzed by using the software Coral Draw 13, Coral Inc. 2005.

#### INDOOR OVITRAP SURVEILLANCE OF MOSQUITO SPECIES IN SELECTED AREAS AT PESHAWAR VALLEY (KHYBER PAKHTUNKHWA)

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SALMAN AND MUHAMMAD NISAR

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Indoor ovitrap surveillance was conducted in the selected areas at Peshawar, Nowshera, Charsadda and Mardan districts of Khyber Pakhtunkhwa, Pakistan during 2012-13 in order to monitor the dengue vector population dynamics and to provide baseline entomological information for devising an optimum vector control program. The results revealed that various species of mosquitoes showed significant variations in abundance collected from different indoor sites. *Culex spp.* dominated the other two species. Highest positive ovitrap index for *Culex spp.* as observed in Mardan (16.05%) and the lowest in Peshawar (9.38%), whereas, for *Anopheles spp.* Maximum ovitrap index (6.57%) was noted at Charsadda and minimum (2.64%) at Mardan. The activities of *Culex* and *Anopheles* started in January, increased up to May, decreased during June-July, peaked during August-September and decreased to minimum till December. The results indicated that *Aedes* mosquitoes appeared in the months of November and December and no *Aedes* were observed in the rest of the months with maximum positive ovitrap index (4.4 %) at Nowshera followed by Peshawar (2.8 %), Charsadda (2.7 %) and Mardan district (2.0 %). Data collected in this study could provide significant entomological information for planning an effective integrated vector control program for management of mosquito's species in the studied area.

**EFFECT OF SEASONAL VARIATION ON ABUNDANCE OF TWO SPECIES OF GENUS ERISTALIS (DIPTERA: SYRPHIDAE) IN HYDERABAD REGION**

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Hoverflies are also called flower flies, syrphid flies or drone flies and move through the air more like flies, zipping from plant to plant, hovering temporarily before landing. These stingless flies are harmless to humans and other animals despite their mimicry of more dangerous wasps and bees, which serves to fend off predators. Effect of seasonal variation on abundance of *Eristalis aeneus* and *Eristalis megacephalus* of genus *Eristalis* was studied in different localities of Hyderabad region during March to December 2015. Adult hoverflies were collected/trapped by different methods i.e. Malaise trap, light trap and insect hand net. The maximum population of these flies was recorded in the month of December, followed by March and minimum population was recorded in the month of May, June and July respectively. These results indicate that abundance of species of genus *Eristalis* was positively correlated with the floral abundance and flowering plant species, while relative humidity and temperature were negatively or only weakly correlated.

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**SONGS PATTERNS IN THE HOUSE CRICKET ACHETA DOMESTICUS (L.) SHOWING SOME SEASONAL AND GEOGRAPHICAL VARIATIONS**

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In the present work specimens of *Acheta domesticus* were collected from different areas of Karachi and their songs were recorded and were analyzed with reference to their different song patterns i. e. chirp rate, pulse rate, inter pulse interval (IPI), inter chirp rate (ICI), chirp frequency, chirp duration and band energy and used these characters for the taxonomy of house crickets. *A. domesticus* (L.) was described from Pakistan by Ghouri (1961) and described its imaginary distribution in South-western Asia and Africa. Kalifa (1950) stated that *A. domesticus* is now became cosmopolitan. In cricket group the external morphological characters including male and female genitalia are not considered as successful identifying taxonomic characters. Allard (1910) noted the Geographical variations in song patterns whereas Fulton (1957) was the first taxonomist who used the songs as their taxonomic character to separate cricket species including their sibling species. Alexander (1957) presented the sonogram of *A. domesticus*. Weissman and Rentz (1977) redescribed its sonogram with a few characters. Now with the help of new recording technologies, acoustic character is considered as reliable character for their identification. Specimens of *A. domesticus* were collected from different areas of Pakistan. Songs of male specimens were recorded by Zoom H4 next recorder and slices were prepared by using Audacity portable Digital Audio Editor Version 1.2.6. These sounds were studied and analyzed by the use of software Praat 5400\_win 32. These songs were observed from different areas and in different seasons to analyze the variations.

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**BIOLOGY OF *AILOPUS THALASSINUS THALASSINUS* (ACRIDIDAE: OTHOPTERA)  
FROM TAULKA K. N SHAH DISTRICT DADU**

NAHEED BALOCH<sup>1</sup> AND N.M.SOOMRO,<sup>1</sup> ABDUL SATTAR KALHORO<sup>2,\*</sup> AND ASIF NAZEER<sup>2</sup>

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The genus *Aiolopus thalassinus* represents one of the most remarkable member of the order orthoptera, *Aiolopus thalassinus* is reported as a major pest of rice, sugar cane, wheat, maize & alfa alfa during the present study. Its important life parameters i.e., mating, copulation, oviposition and development of different instars were studied. A total 380 specimens including both mature and immature were collected from different localities of taulka K N Shah district Dadu such as village Saindad Lakhair, village, kalhoroa, village Ariz Muhammad Gadhi, village Sita, K.N Shah city and village Kollachi during the year 2014-2015.

**TAXONOMY AND ECOLOGY OF (OXYINAE ACRIDIDAE: OTHOPTERA) FROM  
DISTRICT DADU & JAMSHORO**

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The Sub family *Oxyinae* represents one of the most remarkable member of the order orthoptera, is reported as a major pest of rice, sugar cane, wheat, maize & alfa alfa minor pest of corn, gram plants, and vegetables during the present study. Its important parameters i.e size, structure, coloration, measurement of body parts, and ecological aspects were studied. A total 1600 specimens including 07 species were collected from different localities of taulka & district Dadu and such as village Lal bux Panhwar, village Makhdoom Bilawal, village Bhawalpur, Village Sita, Village Maloja Panhwar and village Imam Ali Dal, village Haji Paryal Shah, Bhan Syedabad town, village Bakhtyar Pur and adjoin area of Sehwan of taulka Sehwan Shareef District Jamshoro during the year 2015.

**TAXONOMIC STUDY OF STINK BUGS (HEMIPTERA, PENTATOMIDAE, HALYINI)  
OF PAKISTAN**

ABDUL MANAN SHAIKH,<sup>1</sup> NASREEN MEMON,<sup>2</sup> MANSOOR ALI SHAH<sup>2</sup> AND NADIR ALI BIRMANI<sup>2</sup>

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Taxonomic studies of Halyine species has been conducted from all four provinces of Pakistan during 2004 to 2010. Collected material has been identified with the help of available literature and

preserved material including Holotypes and paratypes. Keys of different genera and different species of tribe Halyini has been prepared and modified.

#### REVISION AND NEW RECORD OF (HEMIPTERA: PENTATOMIDAE) DISTRICT HYDERABAD AND ADJOINING AREAS

RESHMA SAHITO,<sup>1</sup> NASREEN MEMON,<sup>2</sup> ABDUL MANAN SHAIKH,<sup>3</sup> DILDAR ALI SOLANGI<sup>4</sup> AND BHOJOO MAL<sup>5</sup>

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Pentatomidae is one of largest families of order Hemiptera. It is famous with the name of stink bugs because they produce bad smell. They constitute an economically important group of hemiptera pests as most of species are phytophagous both nymphs and adults have sucking and piercing type of mouth parts. They suck the sap by piercing their rostrum into plant tissues and lower its vitality from plants they also transmit bacterial, viral and fungal diseases. In the result of extensive survey a total of 363 specimen were collected from March to July 2015, with the help of insect net, light trap and handpicking method from localities Jamshoro, Matiari, Tando Jam, Tando Mohammad Khan from district Hyderabad and its adjoining areas. All collection was preserved according to standard method and identification of genus and species, by using taxonomic keys and literature on the basis of morphological characters. Two species were identified belong to genus *Brachynema*, *B. virens*, and *Coenus*, *C. delius*. identification was done by using binocular on the basis of morphological description along with measurement of different body and compare the species with the relevant. These species are newly identified in Hyderabad.

#### AQUATIC INSECTS OF GUJRANWALA

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The present work deals with the systematic account of aquatic insects of Gujranwala. During this survey the aquatic insects were collected from different lentic and lotic water bodies of various sizes. The material was pinned and stored in insect boxes. This short term investigation has revealed a total of 9 families, 21 genera and 23 species of Hemipteran and Coleopteran aquatic insects. Their breakdown is as follows: Order Hemiptera: Family Corixidae: *Corixa substriata*, *Micronecta proba*, *M. thyesta*, Family Naucoridae: *Ambrysus* sp.; Family Nepidae: *Nepa ruber*, *N. elongates* and *Ranatra filiformis* and family: Notonectidae: *Anisops sardea*. Order Coleoptera: Family Dytiscidae: *Dytiscus* sp., *Eretes* sp., *Hyphydrus* sp., *Hydrovatus* sp., *Ilybius* sp., Family Hydraenidae: *Octhebius* sp., Family Hydrophilidae: *Berosus* sp., *Helochaers* sp., *Hydrophilus* sp., *Laccobius* sp., *Philydrus* sp., *Tropisternus* sp. and Family Noteridae: *suphisellus lineatus*



**NEW RECORD OF DRAGONLIES TWO SPECIES OF GENUS ORTHETRUM  
(ODONATA: LIBELLULIDAE) IN DISTRICT KHAIRPUR MIR'S SINDH**

SAJIDA MEMON,<sup>1</sup> NASREEN MEMON,<sup>2</sup> ABDUL MANAN SHAIKH,<sup>1</sup> BHOJOO MAL<sup>1</sup> AND  
DILDAR ALI SOLANGI<sup>2</sup>

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Orthetrum Newmen, (1833) genus of dragonflies in family libellulidae it is a very large genus includes 61 species found throughout world Dragonflies belong to order Odonata (toothed ones) and suborder Anisoptera (biting and chewing type of mouth parts) is the most beautiful insects that ever roamed the earth. These are important fauna as predator and prey as well as involve in control of several epidemic diseases like Malaria, Filariasis and Dengue fever etc. present study of dragonflies will be helpful for the general public and health, agricultural department to control certain crop pest which lose our economy and also control malaria and Filariasis. During present survey a total 250 of specimens were collected during the months of March, April, May, June, July, August 2015 from different localities of district Khairpur Such as Gambat, Pir-jo-Goth, Khuhra and Kotdiji specimens Identification has been done by the available literature diagnostic characteristics body coloration, wing venation and primary genital organs. Two species of genus Orthetrum, *O. Sabina*, *O. chrysis* were identified through relevant literature and standard method. These species was first time recorded from District Khairpur Mir's Sindh, Pakistan.

**FITNESS OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*, ON DIFFERENT  
FLOUR TYPES**

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The red flour beetle is a cosmopolitan insect pest and feeds on a variety of cereal grains and flours. It is a common problem in flour mills where a variety of flours are present. The use of insecticides for the management of the red flour beetles is hazardous because insecticidal applications in food industries may contaminate food items and usually have long lasting residual effects which ultimately create public health concerns. To design a non-chemical management strategy, food preference of the red flour beetle was studied on different types of flours (whole wheat flour, white wheat flour, corn flour, semolina flour, rice flour, gram flour). Eggs of the beetles were introduced in each type of flour and different life history parameters were studied: larval and pupal duration, survival rate of immature and adult stages and fecundity. The developmental time was shorter and survival rate was better on whole wheat flour than rest of the flours. The study concludes that in food industries where different types of flours are kept in stores for further processing, special emphasis should be given to whole wheat flour for the management of the red flour beetles.

### TAXONOMIC STUDY OF *POLISTES* FAUNA (VESPIDAE: POLISTINAE) MANSEHRA, PAKISTAN

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During the present study *Polistes* species were collected from District Mansehra for the present study. The identified specimens were belonging six (6) species *Polistes indica*, *Polistes olivaceous*, *Polistes stigma*, *Polistes wattii*, *Polistes rothneyi* and *Ropalida brevita* in two (2) genera *Polistes* and *Ropalidia* of subfamily *Polistinae*. Among these *R. brevita* is new record for this area. It was also observed that specimens of species *Polistes wattii* were abundant in the area and were found as predatory on fruit crops which play as pollinators in the area.

### DESCRIPTION OF ONE NEW SPECIES *MICROTHESPIS ODERAI* (RIVETINAE, MANTIDAE, MANTODEA) FROM SINDH, PAKISTAN

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The *Microthespis oderai* n. sp. has been described as a new work from Sindh, Pakistan.

### MASS REARING OF *CHRYSOPERLA CARNEA* BY USING DIFFERENT FILLER MATERIAL UNDER LABORATORY CONDITIONS

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To avoid cannibalism in *Chrysopid* larvae, several studies have been undertaken by the researchers and Entomologists for cheapest insectary through different techniques. Therefore present study was designed to reduce cannibalism in *Chrysoperla Carnea* larvae by using different filler materials under laboratory conditions. Four different filler materials viz: wood shave, saw dust, packing material, plastic crystal straws pieces and control (plastic tube). The observation was recorded on egg survival, larval period, larval survival, pupal weight and pupal period for parents up to three generations. The results revealed that maximum egg survival ( $86.50 \pm 2.90$ ) was recorded in control in parents as compared with generation one, two and three, followed by wood shave, saw dust and packing material. Whereas, minimum egg survival ( $43.00 \pm 2.48$ ) was recorded in plastic straw pieces. The highest larval survival ( $73.00 \pm 2.48$  and  $67.00 \pm 3.93$ ) respectively was recorded in control and wood shave. While minimum larval survival was recorded in plastic straw pieces ( $20.00 \pm 2.48$ ). Furthermore, maximum pupal weight ( $0.07 \pm 1.79$ ) was observed in control and

minimum pupal weight ( $0.03 \pm 2.06$ ) in plastic straw pieces. The reduced larval period ( $7.00 \pm 0.25$ ) was observed in control and longest ( $10.50 \pm 0.64$ ) in plastic straw pieces. Similarly, the shortest pupal period ( $4.25 \pm 0.25$ ) was observed in control and higher pupal ( $7.75 \pm 0.25$ ) in plastic straw pieces. The results of this study would be helpful for the mass rearing of *C.carnea* on large scale for the management of noxious pests in variety of crop.

**BIOLOGY OF A PRAYING MANTID TENODERA ATTENUATTA (STOLL),  
(MANTODEA: MANTIDAE: MANTINAE) FROM SINDH**

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Specimens and oothecae were collected during the survey of Sindh province in year 2009-2013. *Tenodera attunata* (Stoll) was sorted and selected for rearing in laboratory and green house. Its oviposition, hatching, feeding and mating was observed.

**ALTERNATE DIETARY SOURCES FOR COCCINELLID LADYBIRD BEETLES IN THE  
LABORATORY**

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Laboratory rearing of ladybirds often need live aphids. Short seasonal availability of aphids hinders the rearing procedures. We compared the longevity and egg laying of two different species of ladybirds viz., zigzag and seven spotted on alternate foods. Ladybird beetles survived for substantial period of time on alternate food like frozen eggs, frozen aphids, soaked raisins or combination of these but the egg laying was affected. The natural diet comprising aphid host proved significantly superior to the alternate diets. Zigzag beetle produced few eggs on frozen egg diet. Seven spotted ladybird beetle survived for almost a month on simple sugar or honey solutions.

**LADY BIRDS BEETLES FAUNA FROM KURRAM AGENCY OF PAKISTAN**

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Lady birds beetles fauna inhabiting Kurram agency of Pakistan was studied during April 2014 to November 2014 in 20 localities. Out of 13 collected species under 9 genera, 9 species were

found to be new addition from study area. The new additions to the area were *Chilocorus bijugus*, *Coccinella transversoguttata*, *Hormonia diamiata*, *Illeistim berlakei*, *Aiolocaria hexaspilota*, *Menochilus sexmaculatus*, *Oenopia mimica*, *Halyzia tschitscherini*, and *Henospilachina vigintioctopunctata*.

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**DETERMINATION OF ECOLOGICAL DIVERSITY OF ORDER DIPTERA, COLEOPTERA AND SIPHONAPTERA AMONG OKRA (ABELMOSCHUS ESCULENTUS L.), POTATO (SOLANUM TUBEROSUM L.) AND CAULIFLOWER (BRASSICA OLERACEA L.) FIELDS**

SOMIA AFZAL, NAUREEN RANA, MUHAMMAD ZAFAR IQBAL, RUBAB ZULIFQAR, SEHAR JAHANGIR, YUSRA YASMIN AND SAIMA SIKANDAR

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Present study was conducted to determine ecological diversity of order Diptera, Coleoptera and Siphonaptera among okra (*Abelmoschus esculentus* L.), potato (*Solanum tuberosum* L.) and cauliflower (*Brassica oleracea* L.) fields during the session 2014-15. After completing the research trial with sweep net, direct hand picking and with forceps, total 1148 specimens were recorded and identified up to species level from three vegetable fields. However, as per findings of the previous researchers, population dynamics for order Siphonaptera was recorded nil among three vegetable fields over the entire study period. The relative abundance was maximum in okra fields for order Diptera (65.93%) and lowest for order Coleoptera (34.07%). However, in potato fields relative abundance was at peak for the order Coleoptera (68.37%) and least for order Diptera (30.95%); whereas in cauliflower fields, maximum relative abundance was recorded again for order Coleoptera (69.05%) and minimum for order Diptera (30.63%). For order Diptera *Physiphora* genus 77.50% ( $n \geq 93$ ), *Fannia* 29.03% ( $n \geq 54$ ) and genus *Eristalinus* 25.64% ( $n \geq 30$ ) were documented at peak in Okra, Potato and Cauliflower fields respectively. For order Coleoptera, in Okra genus *Myloccerus* (Family Curculionidae) 50.00% ( $n \geq 31$ ); genus *Psammodes* (Family Tenebrionidae) 30.27% ( $n \geq 79$ ) in Potato and *Sitona* (Family Curculionidae) 45.52% ( $n \geq 183$ ) in Cauliflower fields were recorded at peak. The results of ANOVA between depending variables (Okra, Potato and Cauliflower) and independent variables (Coleoptera and Diptera) were not convincing the imperative status of significance ( $F= 1.53$ ;  $P= 0.2175$ ). The results of Kruskal-Wallis test pertaining to rank order of taxa composition and population density were much convincing and significant ( $F = 4.53$ ;  $P= 0.0115$ ). According to Wilcoxon Rank Sum Test, in okra, potato and cauliflower fields population of both orders was differ significantly ( $P$ -value = 0.0007), ( $P$ -value = 0.0001) and ( $P$ -value = 0.0014), respectively.

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**A NEW RECORD OF HOVERFLY SPECIES *MESEMBRIUS BENGALENSIS* (SYRPHIDAE: DIPTERA) FROM KHAIRPUR, PAKISTAN**

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Hoverflies or flower flies are very important insects because of their multiple services in ecosystem. They are moderate to large-sized and brightly coloured. They are worldwide distributed

except Antarctica and consist of about 6000 recognized species. Their larvae show various feeding habits like aphidophagous, saprophagous and phytophagous. Whereas, adults feed on nectar for energy and pollen for proteins, lipids and vitamins. From ecological point of view, the species of Eristalinae act as important pollinators clean the environment as decomposers and help in biological control of crop pests. *Mesembrius bengalensis* is first time recorded from the district Khairpur as well as Sindh. The research studies were conducted from Jan to July 2015 in various districts of Sukkur Division. This newly recorded species was collected during the months of May and June (2015) from Pir-Jo-Goth and Ahmedpur respectively on the host plants Toori and Bittergourd. It has vertical yellow and black bands on thorax and horizontal yellow-black stripes on abdomen, which mimic honeybees. Maximum population of this species was collected in May (18 specimens: 10 ♂ and 8 ♀). Whereas, minimum population was trapped in June (12 specimens: 6 ♂ and 6 ♀). Their presence in these hot months, where temperature was up to 46°C, indicates that they are more prevalent during summer season.

#### TAXONOMIC STUDY OF ANTS FROM DISTRICT MANSEHRA

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Ants of the district Mansehra, Pakistan were explored during 2012-2013 from eighteen selected localities. Out of 277 collected specimens 30 species were identified under 18 genera of three subfamilies namely Camponotinae, Myrmecinae and Ponerinae. Subfamily Myrmicinae was dominant with 22 species under 12 genera followed by subfamily Camponotinae with 7 species under five genera while a single species from subfamily Ponerinae has been identified. Among 30 identified species twelve species were new record for Pakistan.

#### PHYLOGENY OF THE SPECIES OF PALAEARCTIC GENUS *PARANEVISANUS* DISTANT (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE: HALYINI)

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The Palearctic genus *Paranevisanus* is widely distributed in Himalayan range, from Gilgit (north), Muree (West), Kashmir, Simla, Almora, Nani Tal (north west) to Nepal (north east). The species of genus *Paranevisanus* were included: *annandalei* (Distant), *pilipes* (Horvath), *melania* (Distant), *melania kumaonensis* Ghauri, *amberinus* Ghauri, *amberinus jhellum* Ghauri. We examined the phylogenetic relationship among these species by using adult morphological characters including external and internal male and female genital characters. The characters were compared with those found in the out group within the tribe. A cladogram is constructed on the principle of parsimony. We have chosen genus *Apodiphus* as an outgroup, especially on the basis of

resemblance in some distinguish characters like paraclypei with broad apex and smooth lateral margin, mottled body colour and presence of 4-5 pale spots on anterior part of pronotum, but *paranevisanus* has very distinct position in the tribe hlayini because of special structure of male paramere and female spermathecal bulb, which are not only different from outgroup genus *Apodiphus* but also from rest of halyine species.

**MORPHOGENETIC CHARACTERIZATION OF *ANAX IMMECULIFRONS* AND *ANAX PARTHENOPE* (AESHNIDAE: ODONATA) USING 16S RRNA AND CYTOCHROME OXIDASE 1(CO1) GENES**

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A total of 25 samples of *Anax immeculifrons* and 20 samples of *Anax parthenope* were collected from District Mansehra, Batagram and Kohistan, Pakistan during 2011 to 2013. Taxonomic keys were developed to the species level. Phylogenetic tree was reconstructed using 16S rRNA (16S) gene showing homology of 83% compared with gene bank data while on the basis of Cytochrome Oxidase 1 (CO1) gene 95% homology were recorded. It was concluded that CO1 gene is more reliable than 16S rRNA gene for confirmation of *A. immeculifrons* and *A. parthenope* position in the genus *Anax* of Aeshidae family.

**THE BIOGEOGRAPHY OF PIERID BUTTERFLIES (LEPIDOPTERA: PIERIDAE) IN POTOHAR REGION OF PAKISTAN**

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Pierid fauna inhabiting Potohar region of Pakistan was studied during January 2012 to February 2015 along with data collection on its bio-geographical affiliation. Out of 19 collected species, ten species of six genera and nine species of four genera fit in subfamilies Pierinae and Coliadinae. In subfamily Coliadinae, genus *Gonepteryx* and *Colotis* with its species namely *G. mahaguru* and *G. rhamni*, and *C. amata*, *C. etrida*, *C. protactus* while in subfamily Pierinae, genera *Belenois*, and *Delias* with their species *B. aurota* and *D. eucharis* were new records for Potohar region. Data recorded for biogeographic affiliation indicates representation from all biogeographic regions. However, the Afrotropical and the Aust-oriental elements were observed to be dominant.

**DISTRIBUTIONAL DYNAMICS AND OCCURRENCE STUDY OF PREYING MANTIS (INSECTA: DICTYOPTERA) IN QUETTA AND PISHIN DISTRICTS, BALOCHISTAN**

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A research survey was conducted over two years (2013-2014) to investigate the distribution and occurrence of predatory insect (Mantidae) in Quetta and Pishin districts. Specimens of preying mantis were collected from six main areas: Orak valley, Hana, University of Balochistan, Hazar Ganji National Park, Chasma Achozai and Kuchlak of district Quetta, whereas in Pishin district sampling was made from Pishin town and its interior namely Bostan, Mughtiyar, Khanozai, Saranan and Barshore as well. The identification of collected specimens revealed six genera belonging to same tribe Mantodea and single family Mantidae prevailed in these areas. The identified species include *Tenodera aridifolia*, *Rivetina fraterna*, *Iris oratoria*, *Stagmomantis carolina*, *Humbertilla indica* and *Heirodula patelifera* are first time recorded from Balochistan. The species *Stagmomantis carolina* is recorded for the first time from Pakistan and is, therefore, a new addition to mantis fauna of Pakistan. The most encountered and prevailed species was *Tenodera aridifolia* in both districts on vegetations. A large number of specimens were collected from *Pyrus malus* (apple variety), *Medicago sativa* (Alfalfa), *Brassica campestris* (Field mustard), and majority of mantis species were observed feeding on Homopterans such as aphids, scales insects, leaf hoppers and white flies.

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## SECTION – IV

## PARASITOLOGY

**COTUGNIA FLEARI (MEGGIT, 1927) BILQEES 1985, FROM COMMON PASSER DOMESTICUS IN JAMSHORO, SINDH, PAKISTAN**

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During the survey of Helminth parasites eight *Passer domesticus* were shot down from the District Larkana. Out of eight five were found infected from Helminth parasitic infection. Thirty two specimens of Cestodes were recovered from small intestine. These specimens were mounted according to standard procedure for further detail study, and identified belonging to genus *Cotugnia fleari* (Meggit, 1927) Bilqeess 1985. The genus is characterized by having: smaller worms with wide strobilla, scolex is muscular, neck is absent, suckers are larger, each proglottids with two sets of reproductive organs, Vitellaria is compact, immediately post ovarian, Vagina is long and posterior to cirrus sac, testes are on each side, extending in mid-lateral region of the segment post-lateral to ovary and vitellaria, cirrus pouch is cylindrical, vagina is posterior to cirrus pouch, each egg capsule contain single egg.

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**PREVALENCE AND TAXONOMIC SPECTRUM OF HELMINTH PARASITIC INFECTIONS IN FRESH WATER FISH OF RIVER SWAT, KHYBER PAKHTUNKHWA, PAKISTAN**

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Studies on helminth parasites of fish are an important aspect because fish are direct source of food for man. Fish provides primary source of high quality protein in more than one billion people of the world. The public and zoonotic importance of parasitic infection continue due to their prevalence, vitality, distribution and their effects on both nutritional and immune status of population especially those who consume fish as food. A total of 54 fish (*Schizothorax labiatus*) collected from different sites and localities of river swat, Pakistan and were examined in the laboratory for Helminth parasites during the month from February to October 2015. A record of collection, including parasites, host of parasite, location, locality, number of specimen recovered, number of host examined, number of host infected, were kept regularly for reference. Only nematode of the genus *Rhabdochona* was reported. Camera Lucida drawings and photomicrograph were taken for the study in detail. The research data was visualized based on length, weight, sex, month and season. An interesting relationship was noted between body length and prevalence of infection, where length and weight increases, lead to increase prevalence of infection. Female were



found to be highly infected than male fish. Month wise data revealed that the fish trapped in the months of February (33.3%), August (33.3%) and September (33.3%) followed by March (16.6%) were highly infected, while least prevalence rate of infection was reported from the fish collected in the months of May and July (14.28%). No infection was found in the fish collected in the months of April and June. For seasons, (25%) of the prevalence was reported in the fish collected in monsoon followed by (15%) in post monsoon and least prevalence rate (13.3%) was noted for pre-monsoon. Keeping in view that the problem of beef consumption, mad cow diseases foot and mouth disease in sheep and goats, bird flu virus in poultry. There is more demand today for fish than before. Healthy fish (free of parasite disease) is the demand of today and tomorrow. Therefore it is necessary to investigate and highlight the parasitic fauna, their frequency of occurrence and prevalence of infection in order to take steps for the control "elimination" of all these infections.

**NEW SPECIES OF GENUS *RAILLIETINA* (DAVINEIDAE: CESTODA) FROM  
INTESTINE OF COLLARED DOVE *STREPTOPELIA DECAOCTA* (COLUMBIDAE:  
COLUMBIFORMES) FROM SINDH, PAKISTAN**

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During the examination of gut contents and visceral organs of Collared Dove *Streptopelia decaocta* (Columbidae: Columbiformes) from Sindh Region of Pakistan, 15 specimen of genus *Raillietina* Furhman, 1920 (Davineidae: Cestoda) were collected from intestine of three hosts and identified as new species. The specimens dehydrated in graded series of ethanol, stained with borax carmine, cleared in clove oil and xylol and mounted in Canada balsam; diagrams were made through camera lucida. The specimens were found different from its congeners in scolex shape, diameter of sucker, number of hooks arranged in double rows, shape and size of mature and immature segments, number of testes, ovary size and number of eggs. On the basis of these characteristics, a new species *Raillietina decaocta* has been proposed. The name of new species refers to the species name of the type host.

**A NEW RECORD AND NEW SPECIES OF THE GENUS *BRUEELIA* KELER, 1936  
(PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) FROM ZEBRA FINCH  
(PASSERIFORMES: ESTRILDIDAE) FROM SINDH, PAKISTAN**

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Zebra finch, *Taeniopygia guttata* (Passeriformes: Estrildidae) is a captive bird in Sindh region. It is a native of Australian region but successfully established its progeny in other continents of the world. During the present study, zebra finches were observed for their chewing lice in captivity from different regions of Sindh. More than 30 specimens of genus *Brueelia* Keler (Phthiraptera: Ischnocera: Philopteridae) were being collected; mounted permanently in Canada balsam after passing the standard process of dehydration. The specimens were examined under light microscope, drawings, photographs and measurements were taken, and identified as a new species. The specimens were compared with the different species of genus *Brueelia*, reported from estrildid

birds of the world. Differences were noticed in head sutures, tergal and pleural plates, male and female terminalia, subgenital plates and male genitalia. On the basis of these taxonomic characters, the new species is named as *Brueelia rizvii* sp.n. The name of new species is given in the honor of author's respectable teacher, Prof. Dr. Syed Anser Rizvi, for his endless guidance and support.

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**NEW SPECIES OF *BRUEELIA* KELER (PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) FROM SINDH SPARROW *PASSER PYRHONOTUS* BLYTH (PASSERIFORMES: PASSERIDAE) FROM SINDH, PAKISTAN.**

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Sindh sparrow, *Passer pyrhonotus* (Passeriformes: Passeridae) is a common resident of Sindh. It is the first study of Sindh sparrow for its ectoparasites in the region. During the present investigation, fifteen birds were examined from different regions of lower Sindh. The collected specimens of the genus *Brueelia* Keler were permanently mounted in Canada balsam and examined under light microscope for its identification, measurements, chaetotaxy and drawings. Presently, the genus *Brueelia* was reported from this host for the first time from Sindh; ten specimens were studied using updated literature and designated as new species, *B. sindhiensis*. The new species was described and compared with its closest allies in the lice genus *Brueelia* from different hosts of family Passeridae. The taxonomic diagnostic differences appeared between clypeal suture, preantennal nodus, conus, gular plate, subgenital plate and male genitalia. However *Passer pyrhonotus* is the new host, reported from Sindh, Pakistan for the first time.

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**A NEW SPECIES OF GENUS *CENTRORHYNCHUS* (ACANTHOCEPHALA: CENTRORHYNCHIDAE) IN COMMON KINGFISHER *ALCEDO ATTHIS* (CORACIIFORMES: ALCEDINIDAE) IN KHAIRPUR, SINDH, PAKISTAN**

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During current studies on helminth parasites of birds of different feeding habits, 8 acanthocephalan (5♂ and 3♀) belonging to genus *Centrorhynchus* Lühe, 1911 were collected from the intestine of Common Kingfisher *Alcedo atthis* (Coraciiformes: Alcedinidae) of Khairpur district, Sindh, Pakistan. Present specimens differ from their close allies in having more elongated body broader in anterior region; pseudosegmentation present; globular proboscis armed with 23 longitudinal rows of hooks with 9 hooks in each row; lemnisci longer than proboscis receptacle; testes tandem, elongated, situated in anterior wider part of body and four cement glands. On the basis of these diagnostic differentiating characteristics between present and previous known species, a new species *Centrorhynchus atthisi* is proposed to accommodate the present specimen. Name of new species refers to the species name of host bird. Common Kingfisher *Alcedo atthis* is new host record for the genus *Centrorhynchus* Lühe, 1911.

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**NEW HOST RECORD FOR THE *DIPLOTRIAENA MONTICOLAE* (FILARIIDAE: NEMATODA) FROM THE THORACIC CAVITY OF *PASSER PYRRHONOTUS* BLYTH, 1845 (PASSERIDAE: PASSERIFORMES) IN LARKANA, SINDH, PAKISTAN.**

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Five nematodes *Diplotriaena monticolae* Yamaguti, 1935 (02♂, 03♀) belonging to Filariidae: Nematoda were collected from thoracic cavity of the *Passer pyrrhonotus*, killed and preserved in 70% Ethanol and Glycerol solution for detailed study. Diagrams were made with the help of camera lucida and photographs were taken with Nikon digital camera. Measurements are taken in millimeters (mm) and of eggs in micrometers (µm). *Passer pyrrhonotus* is a new host record for the *Diplotriaena monticolae*, this species is reported for the first time from Pakistan.

**DISTRIBUTION OF COMMENSAL RATS AND THEIR HELMINTHS IN DIFFERENT STUDY STRUCTURE OF DISTRICTS MALAKAND AND LOWER DIR KHYBER PAKHTUNKHWA, PAKISTAN**

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Present study was aimed to determine diversity of helminth parasitic fauna of rats and their distribution pattern trapped from various shops of Batkhela, district Malakand, Chakdara and Ouch in district Dir lower, KPK, Pakistan. A total of 53 rats (*Rattus rattus*) were captured from 13 different types of shops (4.07 /shop) using 990 trap nights with a trap success of 0.80. The highest rats population was recorded from Grocery stores of main bazar Ouch followed by butcher shops of main bazar Chakdara, district Dir (lower) while the minimum population was recorded from the sweet shops of main bazar chakdara. Males outnumbered the females. All the rats caught were adult while no juvenile or sub-adult were noted. Season wise highest number of rats were caught in November while lowest in the months of April and May. The rats trapped were examined for the presence of helminth parasitic infection. Two nematodes and one cestodes were recovered while trematodes and acanthocephalan components were totally absent. The overall prevalence was 8 (16%) including 2 (25%) *Syphacia* sp., 1 (12.5%) *Trichuris* sp., and *Hymenolepis* sp. 5(62.5%) were recorded. All the rats were found with single parasite infection, multiparasitism was not reported. Camera lucida drawings and photomicrographs were taken. Present study demonstrate the presence of zoonotically important parasites as like *Trichura* sp. From nematodes and *Hymenolepis* sp. From cestodes which may be transferred from rats to humans. Present study confirmed that *Rattus rattus* is present in all locations in the studied areas. The current study provide information regarding the distribution and abundance of *Rattus rattus* in the studied localities. Further studies are required to understand the impact of these rats on the human life.

**ANAEMIA AND INTESTINAL PARASITIC INFECTIONS AMONG SOME SCHOOL CHILDREN OF DISTRICTS MALAKAND AND LOWER DIR, KHYBER PAKHTUNKHWA, PAKISTAN**

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World Health Organization defines anemia by age, sex, and pregnancy status as follows: for children 6 months to 5 years of age anemia is defined as a Hb level <11g/dL, children 5–11 years of age Hb<11.5 g/dL, adult males Hb<13 g/dL; non-pregnant females Hb<12g/dL; pregnant females Hb<11g/dL and severe anemia is defined as Hb<7.0 g/dL. 3ml of venous blood sample was collected in EDTA container. Of the total one hundred and sixty (160) subjects, twenty seven (27) were females and one hundred and thirty three (133) were males. Intestinal parasite assessment was done by direct smear technique and Formol-Ether concentration methods. Hemoglobin concentration was analyzed using haemometer. Eighty seven (87) subjects were found anaemic and infested with one or more than one species of intestinal parasites. 58 anaemic individuals were found infected with single, 19 double and 05 individuals with triple intestinal parasitic infections. Eight species of intestinal parasites including *Ascaris lumbricoides*, *Trichuris trichura*, *Enterobius vermicularis* and *Ancylostoma duodenale* in nematodes, *Taenia saginata* and *Hymenolepis nana* in cestodes, *Entamoeba histolytica* and *Giardia lamblia* in protozoans were recorded. Overall prevalence was 49 (44.1%) *T. saginata*, 25 (22.5%) *A. duodenale*, 06 (5.40%) *E. vermicularis*, 08 (7.20%) *H. nana*, 02 (1.80%) *T. trichura*, 15 (13.5%) *A. lumbricoides*, 01 (0.90%) *E. histolytica* and 01 (0.90%) *G. lamblia* were reported. Individuals with age 12 – 13 years were found highly infected with association of parasitic infections and anaemia followed by 10 – 11 years while least infections of this association was noted for the age 06 – 09 years. Male students were found highly infected with this association than females. The Mean  $\pm$  Standard Deviation was:  $0 \pm 1.87$ ,  $4.87 \pm 5.64$  and  $7.35 \pm 8.73$  for the ages 06 – 09, 10 – 11 and 12 -13 years respectively.  $0.9 \pm 11.1$  and  $2.25 \pm 2.86$  for males and females. Our data confirm that intestinal parasites are associated with anemia in age and gender in children.

**HISTOPATHOLOGICAL CHANGES IN THE INTESTINE OF INFECTED PIGEON (*COLUMBA LIVIA* GMELIN) NATURALLY INFECTED WITH HELMINTH PARASITES FROM HYDERABAD, SINDH, PAKISTAN.**

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The histopathological studies have been conducted in the present investigation to examine the pathological and parasitic impact in different organs of Rock Pigeon, *Columba livia* Gmelin. The present study was under taken to check out the histopathology caused by Cestode *Raillitena tetragona* Molin, 1858 and Nematode *Ascaridia columbae* Gmelin, 1780 in the duodenum of alimentary canal of the infected bird. During present investigation, 20 rock pigeons were collected randomly from different regions of Hyderabad city. The pigeons were dissected and their alimentary canal were carefully removed and examined for helminth parasites. 12 birds were

infected with cestodes and nematodes. Tissue samples from infected parts of alimentary canal were fixed in Bouin's fluid. The series of sections were made by microtome technique and were mounted in Canada balsam. In infected pigeons, histopathological findings revealed architectural disintegration of muscularis layer, destruction of crypt and Brunner's glands, serosal necrosis, migratory tunnels formed along with fibrosis, villus atrophy and necrosis and infiltration of mononuclear (lymphocytes and macrophages) inflammatory cells in lamina propria were noticed. The random collection of birds showed the medium to high rate of infection which was 60% in the region including 75% cestodes and 25% nematodes were obtained.

**A NEW SPECIES OF GENUS *MYRSIDEA* WATERSTON, 1915  
(PHTHIRAPTERA: AMBLYCERA: MENOPONIDAE) ON *ACRIDOTHERES TRISTIS*  
(PASSERIFORMES: STURNIDEA) FROM HYDERABAD, SINDH, PAKISTAN**

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The genus *Myrsidea* Waterston, 1915 is represented by more than 200 species throughout the world. The genus parasitizes three orders of Aves, including Passeriformes, Apodiformes, Piciformes. The Common Mynah, *Acridotheres tristis* (Linnaeus) (Passeriformes: Sturnidae) was previously reported to harbour one species of *Myrsidea invadens* (Kellogg and Chapman, 1902). In present, the host *A. tristis* was examined for more variety of this genus from Sindh, Pakistan; collected specimens of genus *Myrsidea* were mounted permanently in Canada balsam and studied thoroughly under light microscope for their identification, measurement, and drawing. During the present work, two species of *Myrsidea* were collected from *A. tristis*, *M. invadens* and one new species *M. ahmedalii* from Sindh, Pakistan. Both species were compared for their chaetotaxy, female abdominal tergites and male genitalia. The new species has been named after Dr. Ahmed Ali, father of first author.

***EPISTHMIUM JAMSHORENSIS* SP.N. (TREMATODA: ECHINOSTOMATIDAE) FROM  
THE *EGRETTA GARZETTA* (LITTLE EGRET) IN SINDH, PAKISTAN**

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During survey of helminth parasitic infection in *Egretta garzetta* Little egret were shot down from the District Jamshoro, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infection. Three hosts were examined in random intervals, out of which one was found infected with eight trematodes, these specimens were recovered from small intestine. The specimens were mounted permanently according to standard procedure. For further detail study and identified as belonging to the genus *Episthmium* (Luhe, 1909) *jamshorensis* sp. n. The

new species is characterized by having: Body of worm is flattened, spinose, pulmp shaped, small sized. Head collar is reniform, it bears 28 spines of which 14 are on each lobe, interrupted dorsally in oral region. The oral sucker is terminal, much smaller than ventral sucker. Pre-pharynx is very short, followed by muscular pharynx. Esophagus is long, divided into two blind intestinal caeca. The intestinal caeca are not obvious due to dense distribution of Vitelline follicles posterior to uterus. Cirrus sac is balloon shaped situated above the intestinal bifurcation. The acetabulum is rounded, located at 2<sup>nd</sup> Quarter of the body, much larger than the oral sucker. Ovary is spherical, smaller, pretesticular, closer to acetabulum. Testes are tandem, post ovarian, located in the posterior most region of the hind body. Vitellaria extends from pharynx in the anterior region up to the posterior end of the body, arranged in lateral fields and meet behind the uterus at tip of the posterior region of the body. Uterus is short. Eggs oval to rounded, double walled and larger in size. On the basis of above mentioned differentiating diagnostic characters that the present specimens are new to science, therefore a new species *Episthmium jamshorensis* sp.n. is proposed. The Species name refers to the locality of host bird.

***KNIPOWITSCHIATREMA STERNULAI* SP.N. (TREMATODA: HETEROPHYIDAE) IN  
*STERNULA ALBIFRONS* (LITTLE TERN) IN HYDERABAD, SINDH, PAKISTAN**

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During current studies on the helminth parasites of *Sternula albifrons* (Little tern). A total of five birds were purchased from District Hyderabad, Sindh, Pakistan, out of five one was found infected with two specimens. The worms were mounted permanently according to standard procedure for further detail study and identified as belonging to genus *Knipowitschiatrema* (Issaitschikow, 1927) *sternuai* sp. n. The new species is characterized by having: Body sub cylindrical, anterior and posterior ends broadly rounded. The oral sucker is terminal, broader than long and smaller than the ventral sucker. Pre-pharynx is very short. Pharynx is muscular, rounded and much smaller than the acetabulum. Ceaca terminate at posterior extremity at level of testes. Ventral sucker much larger, rounded and located in the first quarter of the body, Seminal vesicle located far below the ventral sucker, it is elongated, saccular with narrow anterior portion. Ovary is roughly spherical and smaller in size, pre-testicular near middle of the body. Testes tandem, anterior testis smaller and roughly spherical, while posterior testis is larger and rounded in shape. Vitelline follicles commence from the level of testes in the posterior region of the body and arranged laterally. Uterus fills the space below the testes and turns between the testes, ovary and seminal vesicle, reaches above the seminal vesicle to open into genital opening below the acetabulum. Eggs are smaller and double walled. The above facts suggest that the present specimens are new to science hence named as *Knipowitschiatrema sternuai* sp. n. The new species name refers to the host bird.

**PREVALENCE OF *ANCYCLOSTOMA DUODENALE* (HOOKWORM) AND  
*HYMENOLEPIS NANA* (DWARF TAPEWORM) IN CHILDREN OF URBAN AND RURAL  
POPULATION OF LAHORE**

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Parasitic infection is a serious public health problem in the world, especially in the developing countries and represents a major cause of morbidity and mortality in childhood and among high risk groups in most parts of the world. This study was conducted to find out the prevalence of *Ancylostoma duodenale* (Hookworm) and *Hymenolepis nana* (Dwarf tapeworm) in children of urban and rural population of Lahore. We applied three different techniques such as direct smear method, sedimentation technique and flotation technique on 50 samples to find out the prevalence of *A. duodenale* and *H. nana*. All samples were categorized in two groups. 25 samples were taken from urban area and other 25 samples were taken from rural area. Semi structured questionnaire was administered to study subjects. Chi square test was used to find out the prevalence of *A. duodenale* and *H. nana*. No organism was seen in samples from urban area while in rural area 4% samples showed the presence of *A. duodenale* and 4% samples showed that of *H. nana*. The results of study revealed that parents' education had a positive relationship with the prevalence of infection. The effect of hand washing habit was also related to prevalence of infection.

**IMPACT OF CESTODE HELMINTH, *HYMENOLEPIS* SPP. (WEINLAND, 1858) IN  
JUNGLE BABBLER BIRD**

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Jungle babbler, *T. striata* locally called (Sath bhai) in sub-continent of Indo-Pak, which is a common resident breeding bird, often seen in gardens, crops and scrub-jungles lives in gregarious shape of birds which forage in a small group of 5-9 birds and well known as beneficial to cultivated agro-crops. During the course of research work, the overall (n=71) jungle babbler were dissected throughout the year in both summer and winter seasons. This research of dissection, preservation, permanent slides preparation, making diagrams, photography work was conducted at department of Zoology, Shah Abdul Latif University - Khairpur during, 2014. Only (n=95) specimen of genus; *Hymenolepis* were observed. That is known as parasitic cestode belongs to the family *Hymenolepididae*, sub-family: *Hymenolepidinae* and Genus: *Hymenolepis* Weinland, 1858 dwell in the intestine from these birds. From the different review of literature it is identified as; this parasite is different from other species of this genus. This is the first parasite reported from the bird jungle babbler therefore; it should be described as *Hymenolepis mehrabpurensis* (Mangrio and Sahito, 2015) due to its newness reported at taluka Mehrabpur district Naushahro Feroze, Sindh - Pakistan. The specimens of this helminthes parasite are hereby kept under the museum department of Zoology, SALU - Khairpur. It is concluded that, this is a new species of cestode parasite found from jungle babbler the more research work should be conducted to enhance the presence of

## CUTANEOUS LEISHMANIASIS IN IDPs OF WAR AGAINST TERRORISM IN NORTH WAZIRISTAN AGENCY

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## MOLECULAR EPIDEMIOLOGY AND DETECTION OF *FASCIOLA HEPATICA* IN SMALL RUMINANTS OF DISTRICT BANNU, KHYBER PAKHTUNKHWA

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Liver flukes belonging to the genus *Fasciola* are among the causes of food and water borne parasitic disease. The parasites cause significant public health problems and substantial economic losses to the livestock in Pakistan. The study was carried out in District Bannu Khyber Pakhtunkhwa from March to October, 2015 to observe the molecular epidemics and detection of *Fasciola hepatica*. A total of 610 livers and fecal samples were randomly collected from different slaughter houses of sheep and goats. Liver samples were surgically examined for the presence of the fluke, while the eggs identification was carried out in fecal samples by microscopy while the molecular identification of *Fasciola hepatica* was carried out by Polymerase Chain Reaction (PCR). Over all prevalence of *Fasciola* eggs in coprological examination was found to be 11.4%



(n=70/610) in both sheep and goats. Coprology reveals that 7.05% (n= 43/610) samples were positive for *Fasciola* eggs in sheep and 4.42% (n= 27/610) in goats. Livers infected with adult flukes were 4.1% (n=25/610). Individually infected sheep with adult flukes were 2.78% (n=17/610) and goats were 1.3% (n= 8/610). PCR conformed species specific *Fasciola hepatica* infection for all and none for *Fasciola gigantica*. Highest rate of infection 4.42% (n= 27/610) was found in the month of August and lowest 0.82% (n= 5/610) in the month of May. Furthermore, most frequent infection rate was investigated in the weight group of 25 to 50 kg in both sheep and goats. Statistically it was observed that small ruminants were significantly ( $p<0.05$ ) infected with fascioliasis in the studied area.

#### **HISTOPATHOLOGY OF INTESTINE OF FRESH WATER FISH *CHANNA PUNCTATUS* (BLOCH, 1793) INFECTED WITH ENCYSTED METACERCARIAE**

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This study was conducted to know the pathological changes in the intestine of fresh water fish *channa punctatus* naturally infected with encysted metacercariae. During present investigations 75 fishes (*Channa punctatus*) were examined. The parasites collected from the intestine were preserved, stained and mounted by using standard techniques. For the histopathological investigation tissue samples from infected intestine were fixed in Bouin's fluid for 24 hours using routine histological techniques, 6-8 micron thick sections were prepared and stained with hematoxylin and eosin finally mounted in Canada balsam. Histopathology of intestine showed disintegration of villi and lamina propria and inflammatory cells. Encysted parts of parasite found attached with muscular mucosa causing damage to the intestine.

#### **A NEW SPECIES OF GENUS *PHARYPHOSTOMUM* DIETZ, 1909 (TREMATODA: ECHINOSTOMATIDAE) FROM *PHALACROCORAX NIGER* IN PAKISTAN**

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During examination of eleven Little Cormorant *Phalacrocorax niger* for the presence of helminth parasites 25 trematodes were recovered from intestine of five hosts. These trematodes have large, elongated body, reniform head collar equipped with 22 spines, large acetabulum, oval cirrus sac, multilobed testes, spherical ovary, broad uterus and prominent and lateral vitellaria. These were compared with closely related species and found differing from their congeners in number and size of spines, acetabulum, uterus and eggs, shape of cirrus sac, position of ovary and of testes. Therefore, these are recorded as a new to science and named as *Paryphostomum sanghari*. The name of species refers to the locality of host from where it was collected.

**TREMATODES OF CROW, MYNA AND KITE OF DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN**MIAN SAYED KHAN<sup>1</sup> AND SULEMAN<sup>2</sup><sup>1</sup>Department of Zoology, University of Swabi, Pakistan<sup>2</sup>Department of Zoology, Hazara University Mansehra, PakistanEmail: [zaheer0332@gmail.com](mailto:zaheer0332@gmail.com); [zaheer3485@yahoo.com](mailto:zaheer3485@yahoo.com)

During present investigation (July 2012- March 2014) a total of 100 birds were collected at random from all tehsils of District Swabi, KP, Pakistan. Gastrointestinal tract and other different organs like liver, Kidneys and lungs were examined for the trematode parasites. A total of eight species of trematodes were recovered. The trematodes were fixed, preserved, dehydrated, stained and finally permanently mounted in Canada balsam for further study. The microphotographs were taken and the drawings of parasites were made with the aid of a camera Lucida. *Echinochasmus swabiensis* n.sp; *Opisthorchis jonesae* Bilquees and Khan, 2006 and *Neodiplostomum karachiensis* Das and Ghazi, 2011 were reported from black Kite, *Milvus migrans*. *Brachylaima fuscum* Rudolphi, 1819 and *Brachylaima (Brachylaima) sabahense* Fischthal and Kuntz, 1974 from common myna, *Acridotheres tristis* while *Lyperosomum longicauda* Rudolphi, 1809; *Tamerlania swabiensis* n.sp and *Brachylecithum (Brachylecithoides) splendens* n.sp from common crow, *Corvus splendens*. Among them two species; *Opisthorchis jonesae* and *Neodiplostomum karachiensis* are redescribed in Pakistan. *Brachylaima fuscum*; *Brachylaima (Brachylaima) sabahense* and *Lyperosomum longicauda* are new host new locality record while *Tamerlania swabiensis* n.sp; *Brachylecithum (Brachylecithoides) splendens* n.sp and *Echinochasmus swabiensis* n.sp are new to science.

**TREMATODES OF COMMON CROW, CORVUS SPLENDENS OF DISTRICT DIR LOWER, KHYBER PAKHTUNKHWA, PAKISTAN**MIAN SAYED KHAN,<sup>1</sup> KAUSAR ANDALEEB<sup>1</sup> AND SULEMAN<sup>2</sup><sup>1</sup>Department of Zoology, University of Swabi, Pakistan<sup>2</sup>Department of Zoology, Hazara University Mansehra, PakistanEmail: [zaheer0332@gmail.com](mailto:zaheer0332@gmail.com); [zaheer3485@yahoo.com](mailto:zaheer3485@yahoo.com)

*Corvus splendens* is an omnivorous bird feeding on waste foods and on both plants and animals like rodents, reptiles, amphibian and insects. It is therefore exposed to different intermediate host of parasites like trematodes. In the present investigation a total of 83 birds were examined for the presence of trematodes. Three species of trematodes were recovered from the bile ducts (liver) of the host. The trematodes were fixed, preserved, dehydrated, stained and finally permanently mounted in Canada balsam for further study. The microphotographs were taken and the drawings of parasites were made with the aid of a camera Lucida. By using identification keys and available literature, the specimens was identified as *Lyperosomum longicauda* (Rudolphi, 1809) Looss, 1899; *Lyperosomum (Lyperosomoides) petiolatum* Railliet (1900) and *Lyperosomum (Lyperosomoides) pakistanensis* n. sp. Among them one species belongs to the genus *Lyperosomum* Looss, 1899 and two species belong to the subgenus *Lyperosomoides* Yamaguti, 1971. *Lyperosomum longicauda* and *Lyperosomum (Lyperosomoides) petiolatum* are new host new locality records while *Lyperosomum (Lyperosomoides) pakistanensis* n. sp is new to science.

### **HISTOPATHOLOGICAL CHANGES IN THE INTESTINE INDUCED BY NEMATODES PARASITES IN CATFISH, *ARIUS ARIUS* (HAMILTON, 1822) FROM KARACHI COAST**

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This investigation was undertaken to study the histopathological changes in the intestine of catfish, *Arius arius* (Hamilton, 1822) off the Karachi coast due to the nematodes parasites. The sampling was carried out in December, 2014. Infected fishes were purchased from different fish markets and brought to the parasitology laboratory for further detailed investigation by using standard parasitological procedures. Four species of nematodes parasites namely *Raphidascaris acus* (Bloch, 1779), *Metabronema magnum* (Taylor, 1925), *Haplonema immutatum* (Ward et Magath, 1917) and *Hedruris bryttosi* (Yamaguti, 1935) were recovered from the gills and stomach of *Arius arius* (Hamilton, 1822). The histopathological changes include fibrosis, edema, epithelial necrosis, dilation of blood vessels and inflammation.

### **DENDRORCHIS RITATA N.SP. (TREMATODA: GORGODERIDAE) FROM CATFISH *RITA RITA* (SILURIFORMES: BAGRIDAE) OF JAMSHORO DISTRICT, SINDH, PAKISTAN**

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During the course of study conducted for helminth parasites of catfish *Rita rita* of Jamshoro district, a new trematode of genus *Dendrorchis* Travassos, 1926 (Trematoda: Gorgoderidae) is described from intestine of the host fish. The new trematode species is characterized by having small body size; oral sucker terminal; ventral sucker equal in size to oral sucker; testes small, branched, irregular in shape; ovary triangular in shaped, broad posteriorly and narrow anteriorly, situated at left side of body; seminal receptacle present; uterine loops much compact; two vitelline bodies rounded to oval in shape; eggs are oval in shaped. On the basis these diagnostic characteristics, a new species *Dendrorchis ritata* is proposed to accommodate the resent trematode. The name of new species refers to the name of the host fish.

### **INCIDENCE OF TREMATODE PARASITES IN DOLPHINFISH *CORYPHAENA HIPPURUS* (PERCIFORMES: CORYPHAENIDAE) OF GWADAR COAST, BALOCHISTAN**

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Mahi-mahi or common dolphinfish *Coryphaena hippurus* locally called as Aamadosk is among the fastest-growing fishes. Currently 13 fishes were collected from Gawadar cost,

Balochistan, Pakistan for the examination of trematode parasites. All fishes were found harboring the trematodes. Specimens were collected from the liver, spleen, gallbladder and intestine. Intestine was mostly infected with trematode parasites.

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**A NEW TREMATODE *CONSPICUM TRISTITIA* N.SP. (DIGENEA: DICROCOELIIDAE)  
FROM COMMON MYNA *ACRIDOTHERES TRISTIS* L. (PASSERIFORMES:  
STURNIDAE) OF DISTRICT LARKANO, SINDH, PAKISTAN**

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A new trematode *Conspicum tristitia* n.sp. is described from gallbladder of Common Myna *Acridotheres tristis* of district Larkano, Sindh, Pakistan. A total of 5 trematodes were collected and dehydrated in graded series of ethanol. Trematodes were stained in borax carmine, cleared in clove oil and xylol and mounted permanently in Canada balsam. Diagrams were made with aid of camera Lucida. Present trematodes differ from their congeners in body shape and size; presence of shoulder type outgrowths; subterminal oral sucker; wider ventral sucker; presence of testes in parallel to the ventral sucker; pre-equatorial bean-shaped ovary; distribution of vitellaria and post-testicular space. On the basis of these differences, a new species *Conspicum tristitia* is proposed. The name of new species is given after the species name of the host bird.

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***HETERORHABDITIS PAKISTANENSE* N. SP. (NEMATODA: HETERORHABDITIDAE)  
A NEW ENTOMOPATHOGENIC NEMATODE FROM PAKISTAN**

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A new entomopathogenic nematode species of *Heterorhabditis*, described as *H. pakistanense* n. sp., was isolated from grass samples at Malir, Karachi, Sindh, Pakistan. The new species is morphologically characterized by features of males: body size (819; 720-1013), cephalic papillae conspicuous, D% (119; 110-126), SW% (156; 144-191), GS % (58; 48-65) and variations in the number of bursal papillae of the terminal group; 8<sup>th</sup> and 9<sup>th</sup> papillae some time absent on both sides; some time 8<sup>th</sup> papillae present on right side whereas 6 papillae present on the left side. On the right side the arrangement of papillae is 1+2+3+2 whereas on the left side 1+2+3. The hermaphrodites has prominent postanal swelling and a conoid tail (82.3; 64-95)µm long with pointed terminus. Hermaphrodites of *H. pakistanense* n. sp. can be distinguished from all species of *Heterorhabditis* except *H. downesi* by having a mucronate tail. For infective juveniles medium size body (581; 558-624) µm, long pharynx (117; 113-125) µm, ensheathed tail (99; 95-110), and E% (99.9; 95-107). The new species can be distinguished from all species of *Heterorhabditis* by the absence of the 7<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> bursal papillae. *H. pakistanense* is further characterized by the internal transcribed spacer (ITS) and the D2D3 region of the 28S rDNA gene. The closest species *H. indica*, *H. gerrardi*, *H. amazonensis* and *H. noenieputensis* being separated by 9, 7, 66 and 15 bp, respectively in the ITS region. The morphological and phylogenetic analysis confirmed that *H. pakistanense* is a new species of the *indica* group.

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**TWO NEW ENTOMOPATHOGENIC NEMATODE FROM *OSCHEIUS*, *DOLICHURA* GROUP (NEMATODA: RHABDITIDA) AND ONE NEW RECORD AND MOLECULAR WORK OF *O. NAZI* FROM SINDH PAKISTAN**

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Two new species of the genus *Oscheius* were recovered through *Galleria* bait method from soil around the roots of different agroclimatic zone of Pakistan. *Oscheius citrai* n. sp. is characterised by its unique ribosomal DNA18S, sequence, amphimictic reproduction, lateral field with six separate lines, male medium size (1249; 1097-1464)  $\mu$ m, large spicule (average: 63.7  $\mu$ m) and distally with hooked tip, bursa open, peloderan leaving tail spike free, bursal papillae 1++1+3+3. *Oscheius citrai* n.sp. is characterized genetically by the sequence of ITS, KT250509, 872 bp and KR119081.1, 875 bp). *Oscheius sacchari* n. sp. is characterised by its unique ribosomal DNA18S, sequence, amphimictic reproduction, lateral field with six separate lines, greater body width (61.6; 53-75)  $\mu$ m, medium size tail length (35; 32-40)  $\mu$ m, higher b ratio (7; 6.2-8), medium size spicule (50; 44-55)  $\mu$ m and gubernaculum length (20.3; 18-21)  $\mu$ m. In female higher b (8.2; 6.7-10.3) and c (12; 7.6-16.8) ratio. *Oscheius sacchari* n. sp. is characterized genetically by the sequence of ITS, KT250510, 853bp). The morphological and phylogenetic analysis confirmed that *Oscheius citrai* and *Oscheius sacchari* are new species of *dolichura* group.

**HELMINTHIC INFECTION IN HOUSE MOUSE, *MUS MUSCULUS* (RODENTIA: MURIDAE) OF DISTRICT NOSHEHRO FEROZ, SINDH, PAKISTAN**

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The study was conducted to investigate the helminth parasites of House mouse, *Mus musculus* (Rodentia: Muridae) of district Noshehro Feroz, Sindh, Pakistan. For this purpose 23 live hosts were collected from different areas of study area and brought to the Parasitology Laboratory of Department of Zoology, University of Sindh, Jamshoro. During examination of gut contents and visceral organs all hosts were infected with trematodes, nematode and cestodes. The highest prevalence was recorded for the trematodes (82%) followed by nematodes (65%). The minimum prevalence (53%) was recorded for the cestodes.

***ECHINOCHASMUS SINDHENSIS* N.SP. (DIGENEA: ECHINOSTOMATIDAE) FROM THE BANK MYNA *ACRIDOTHERES GINGINIANUS* L. (PASSERIFORMES: STURNIDAE) OF DISTRICT KHAIRPUR, SINDH, PAKISTAN**

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A total of 45 Bank Myna *Acridotheres ginginianus* (Passeriformes: Sturnidae) were examined for the presence of helminth parasites. During examination of gut contents and visceral

organs, 17 live trematodes belonging to the genus *Echinochasmus* Dietz, 1909 were collected from intestines of the hosts. On the basis of shape and size of body; number of collar spines (30); unequal testes, oblique position; arrangement of ovary and testes; vitellaria composed of large follicular groups confluent posteriorly densely arranged in groups throughout the body, commencing from posterior extremity and reaching up to the pharyngeal region, a new species *Echinochasmus sindhensis* n.sp. is proposed to accommodate the present worm. The name of new species refers to the Sindh province from where the host birds were collected.

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**DESCRIPTION OF A NEW TREMATODE *PARAMONOSTOMUM BAGODAROI* N.SP.  
(TREMATODE: NOTOCOTYLIDAE) IN MALLARD *ANAS PLATYRHYNCHOS*  
(ANSERIFORMES: ANATIDAE) IN SINDH, PAKISTAN**

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In result of ongoing helminthological studies on Mallard *Anas platyrhynchos* of Kamber-Shahdaskot District of Sindh province, Pakistan, a total of 57 birds were captured from different localities of study area and brought to the Parasitology laboratory of the department of Zoology, Shah Abdul Latif University Khairpur. During examination of gut contents and visceral organs, a total of 30 specimens belonging to genus *Paramonostomum* Luhe, 1909 were collected from intestine of the host bird. The trematodes were processed according to the standardized parasitological techniques. Present species differs from its congeners on the basis of body shape, more elongated cirrus sac, postbifurcal genital pore, distribution of vitellaria and posttesticular space, a new species *Paramonostomum bagodaro* n.sp. is proposed to accommodate the present worm. Name of new species refers to the locality of host bird.

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***COTUGNIA AASIYASIS* N.SP. (CESTODA: DAVAINIIDAE) FROM DOMESTIC CHICK,  
*GALLUS DOMESTICUS* (GALLIFORMES: PHASIANIDAE) OF DISTRICT KHAIRPUR,  
SINDH, PAKISTAN**

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During current studies on the cestode parasite of *Gallus domesticus* (Galliformes: Phasianidae) of Khairpur district, a total of 50 hosts were examined for the presence of cestode parasites. During examination of gut contents a total of 6 cestodes belonging to genus *Cotugnia* Diamare, 1893 were collected from intestine of single host. Present species differs from its congeners in having scolex and suckers cup-shaped; rostellum armed with hooks; mature segments trapezium in shape; testes 15-20 in number; vitelline gland irregular in shape; gravid segments tetra angular in shape containing round to oval egg capsule with bunches of eggs. On the basis of major diagnostic differences between present species and its close allies, as new species *Cotugnia aasiyasis* is proposed. The name of new species refers to name of late sister of the corresponding author.

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**COMPARITIVE ENDOPARASITIC ANALYSIS AT SELECTED CAPTIVE LOCALITIES  
OF ANTILOPE *CERVI CAPRA* IN PUNJAB**

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Endo-parasites are the Helminths that belong to Cestoda, Nematoda and Trematoda that are common intestinal parasites of Ungulates. They can serve as direct indicators of individual's health. If left untreated could be fatal. We have collected fresh fecal samples from four different sites for the purpose of comparison between different captive and wild conditions i.e. Bahawalpur Zoo, Lahore Zoo, Lal Suhanra National Park (captive and wild). All samples were collected exactly after four days of scheduled deworming. Fecal samples were analyzed for any type of endoparasite infestations under standard lab protocol at Diagnostic Lab Cooper Road Lahore. All samples were positive for endoparasites e.g. tape worms and round worms. Interestingly the black bucks kept at wild conditions were heavily infested by endoparasites despite the fact they were given different natural herbs as de-wormer. Management of all sites was following proper protocols for de-worming but we were not sure that each individual in the herd was taking proper dose of de-wormer. There is need to revise the de-worming protocol otherwise already threatened animal would face more troubles and to spend healthy life style. It is recommended antelopes kept at Lal Sohanra National Park should also be given proper dewormer along with natural wormicides.

***CENTRORHYNCHUS KHAIRPURENSIS* (ACANTHOCEPHALA:  
CENTRORHYNCHIDAE) IN HOUSE CROW *CORVUS SPLENDENS*  
(PASSERIFORMES: CORVIDAE) IN KHAIRPUR, SINDH, PAKISTAN**

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A new species *Centrorhynchus khairpurensis* were collected from House Crow *Corvus splendens* in Khairpur district of Sindh, Pakistan. A total of 11 acanthocephalan (4 ♂ and 7 ♀) of present species were collected from the intestine of host bird. Specimens were pressed under slight cover glass pressure for overnight. Specimens were then stained with borax carmine, dehydrated in graded series of ethanol, cleared in clove oil and xylol. Specimens were finally mounted permanently in Canada balsm. Diagrams were made with help of camera Lucida. On the basis of differentiating characteristics including body shape and size, number of longitudinal rows of hooks, number of hooks per row, shape and position of testes and size of eggs, a new species *Centrorhynchus khairpurensis* is proposed to accommodate the present worm.

**PREVALENCE OF HELMINTH PARASITES OF BOWFIN *AMIA CALVA* L.  
(AMIIFORMES: AMIIDAE) IN SUKKUR, SINDH, PAKISTAN**

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Studies on the prevalence of helminth parasites of freshwater fish Bowfin *Amia calva* L. (Amiiformes: Amiidae) was conducted in Sukkur district, Sindh, Pakistan. During this ongoing study 17 Bowfins were collected and examined. Among these helminth parasites including only trematodes and nematodes were collected. The prevalence of trematodes was recorded highest (71%), whereas of nematodes (65%) was recorded.

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**FIRST RECORD OF GENUS *ECHINOCHASMUS* DIETZ, 1909 IN *CORVUS SPLENDENS*  
(PASSERIFORME :CORVIDAE) IN HYDERABAD SINDH PAKISTAN.**

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During present study on helminth parasites of *Corvus splendens* five trematodes of genus *Echinochasmus* were collected from intestine of single host. They have small, spinose, muscular body attenuated anteriorly, rounded posteriorly; head collar reniform armed with 24 spines arranged in single row, out of them 4 form corner group; oral sucker protruding; ovary spherical, submedian; testis unequal, wider than long contiguous located in posterior half of body; vitellaria compact, non confluent posteriorly. These specimens were compared with related species but closely resemble with *E. bagulai* Verma, 1935 except few minor differences. This is first record of genus *Echinochasmus* from *Corvus splendens*.

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**PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG SCHOOL  
CHILDREN IN TEHSIL-E-MATTA DISTRICT SWAT, PAKISTAN**

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Present study was aimed to determine the prevalence of Intestinal parasitic infections among primary school children in Tehsil-e-Matta, district Swat, Pakistan. The stool specimen, were collected from three government schools including: Government Primary schools Chuprial Khona Cham, Government primary school Gwalera. Government Primary schools Onra Deherai, Tehsil-e-Matta, Swat, Khyber Pakhtunkhwa, Pakistan. Research data was collected through a structured questionnaire by interviewing the students, their parents, guardians and teachers. The stool samples were collected through standard procedure and techniques, All the stool specimen were brought to the Laboratory of Parasitology, Department of Zoology, University of Malakand for parasitological examination. A total 100 students with majority of males were participated in the study and



convinced to give stool samples. Out of the examined 77 (77%) were found infected with one or more than one species of parasites. The pattern of infection reveals that 61 (79.2%) single, 15 (19.4%) double and 1 (1.29%) with triple infections were recorded. The overall prevalence was reported in order of their prevalence as: *Ascaris lumbricoides* 58 (61.7%), *Trichuris trichura* 17 (18.0%), *Hymenolepis nana* 7 (7.44%), *Taenia saginata* 4 (4.25%), hookworms and *Entamoeba histolytica* 3 (3.19%) and *Enterobius vermicularis* 2 (2.12%). The individuals of age group 13 -15 were found more infected followed by 5 - 8 and then 9 - 12. Males were highly infected than females. Present study was laid in its approach to tackle a significant issue, which is the health of the human population of this area in particular and of Pakistan in general. On the basis of the findings of the present investigation it was concluded that the study area is found to be provide supreme opportunity to survive these infections against which we have no satisfactory defence, multiple infection highlight the need of thorough investigation, further studies are required on the same areas.

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**PSEUDOCYATHANEA FORSTERI N.SP. (MONOGENEA: CYATHANEAIDAE LABEDEV, 1972 FROM THE FISH SPHYRAENAE FORSTERI OF KARACHI COAST PAKISTAN**

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Monogenea are primarily fish parasites particularly found on gills and external surfaces. *Pseudocyathanea forsteri* a new monogenea is reported here from the gills of fish *Sphyraenae forsteri*. The new monogenean species characterized by elongated, anteriorly tapered body. Head truncate at apex with terminal mouth, globular pharynx. Esophagus bifurcating in front of vagina. Intestinal limbs subdivided into lateral branches. Numerous rounded testes. Ovary is in the middle fifth of the body far anterior to testes. Spindle shape uterus. Vagina bell-shaped, haptor in V-shaped, asymmetrical without terminal anchors. Clapms are unequal on both sides, 31-37 on right side, 14-20 on left side. The name *Pseudocyathanea forsteri* refers to the host species.

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**PREVALENCE OF SCABIES IN KARACHI, SINDH, PAKISTAN**

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Scabies or the classical itch is caused by the mite *Sarcoptes scabiei* var. *hominis*. Being the most common ectoparasitic dermatosis seen in clinical practice. It is not life threatening condition yet it may be considered important from public point of view because of many reasons that include severe itching which is extremely distressing and in some cases it may become complicated by post-streptococcal glomerulo-nephritis. One year data was collected from the Institute of Skin diseases Karachi. A total of 2,342 patients were observed during the period of investigation (January 2011 to December 2011). 680 patients had scabies while the rest 1662 were infected with various skin diseases which included acne, eczema, pigmentary disorders, urticaria, sweat gland disorders, perioral dermatitis, drug reaction, viral fungal and bacterial infections. The patients adult males and females from different professions as well as children. Treatment with scabicides appeared to be a natural solution, among permethrin and lindane were most effective.

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**ECTO-PARASITE INFESTATION IN PET PIGEON**

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Present study was conducted to determine ecto-parasite infestation in Pet pigeons at Umerkot city from March 2013 to August 2013. Study was carried out in fourteen different areas of Umerkot city. Total hundred 100 pet pigeons (51 Male & 49 Female) were examined physically at pigeon owner houses in Umerkot city and pigeons were found to be infested with two lice species i.e. *Columbicola columbae* & *Menopon gallinae*. The overall prevalence of the lice infestation was recorded as 80% (80/100). The highest infestation rate was recorded in the area of Oad Mohalla and Kolhi Daro 100% (5/5) followed by Sanbhwani, Babar, Khosa Mohalla with the infection rate as 80% (5/4) and Chandiram Mohalla 80% (10/8), in Pathan Mohalla the infestation rate as 70% (10/7), in Machi and Lohar Mohalla the infestation rate was 60% (5/3) and in Sheikh Mohalla infestation rate was also 60% (10/6) respectively. Female pigeons were found in more susceptible with highest infestation rate 85.71% (42 / 49) as compared to 74.50% in males. Severity of infestation was recorded as 60%, 28.75%, and 11.25% as low, medium and high infestation respectively.

**SURVEY OF ECTO-PARASITIC INFESTATION AND ASSOCIATED CONDITIONS IN LIVESTOCK AT TANDO ALLAHYAR ANIMAL MARKET**HAJI KHAN KALOI<sup>1</sup>, BACHAL BHUTTO<sup>1</sup>, ABDULLAH ARIJO<sup>1</sup>, AIJAZ ALI KHOOHARO<sup>2</sup><sup>1</sup>*Department of Veterinary Parasitology, SAU Tandojam, Pakistan*<sup>2</sup>*Department of Statistics, SAU Tandojam, Pakistan*

Survey of ecto-parasitic infestation and associated conditions in livestock was conducted to determine ecto-parasite diversity and to record induced conditions in infested livestock at Tando Allahyar animal market from August 2014 to November 2014. A total of 1500 animals; 600 small ruminants and 900 large ruminants were examined physically in 10 visits at Tando Allahyar animal market. Out of examined, 567 (prevalence  $\pm$  95% CI= 37.80%  $\pm$  2.45) had ecto-parasites. All the four species; cattle, buffalo, sheep and goat; were infested for ecto-parasites. There was (prevalence  $\pm$  95% CI=) 39.69%  $\pm$  5.36, 28.79%  $\pm$  3.68, 48.01%  $\pm$  7.2 and 44.33%  $\pm$  4.78 in cattle, buffalo, sheep and goat respectively. Proportion of ecto-parasitic infestation in animal species were significant at  $P < 0.05$ . There were (prevalence  $\pm$  95% CI=) 42.60%  $\pm$  4.33, 32.60%  $\pm$  4.11, 38.20%  $\pm$  4.26 animals were determined positive for ecto-parasites in tahsil/taulqa Tando Allahyar, Chambar and Jhando Mari respectively. The highest prevalence of ticks (48.40%) than lice (35.65%) and mange (15.14%) was recorded. Furthermore, animals were mostly infested with ticks and lice (64.41%). Fleas were not encountered during the physical examination of the animal's body. Tick species; *Hyalomma*, *Rhipicephalus* and *Boophilus*, lice species; *Bovicola* and *Haematopius* and mange species *Sarcoptes* were found in the study area. In the age groups, cattle calves  $>1$  -  $\leq 5$  years (38.67%) were mostly susceptible than  $\leq 1$  year (22.86%) and buffalo calves  $>1$  -  $\leq 5$  years (27.49%) than  $\leq 1$  year (14.89%). The study also revealed that the cattle  $>5$  years were mostly susceptible (prevalence  $\pm$  95% CI=) 45.19%  $\pm$  8.4 than buffalo 32.27%  $\pm$  5.46. In sheep;  $>1$  year, the ecto-parasitic infestation was significantly ( $P < 0.05$ ) higher (54.61%) than goat (48.66%). The overall

gender-wise ecto-parasitic infestation (prevalence  $\pm 95\%$  CI) in female animals was higher (40.58%  $\pm 2.7$ ) than male (22.51%  $\pm 5.39$ ). The infestation of ticks was higher (46.11%) in Tando Allahyar than Jhando Mari (29.34%) and Chambar (24.55%). Similarly 44.72%, 22.76% and 32.52% infestation of lice was found in Tando Allahyar, Chambar and Jhando Mari, respectively. Beside this, there was 29.09%, 32.73% and 38.18% infestation of mange in Tando Allahyar, Chambar and Jhando Mari, respectively. Proportion of tahsils-wise prevalence of single ecto-parasitic infestation were significantly different ( $P > 0.05$ ). In macroscopic skin disorders skin damages (41.80%) were most frequently found than irritation (22.40%) and dermatitis (9.35%). Prevalence of ecto-parasitic infestation was significantly ( $P > 0.050$ ) different in cottage (36.34%), open (43.60) and cemented type of animal housing (23.39%). Bony animals were most susceptible (48.67%) than fair (38.03%) and fatty (27.22%). There was statistically ( $P > 0.050$ ) significant difference in fair, fatty and bony body status. The infestation of ecto-parasites was found very low (59.08%), whereas, there was 31.57% medium and 9.35% high. Literacy rates found in the study area were 41.00%, 14.67%, 13.00% and 5.00% of primary, matric, intermediate and graduation, respectively. Whereas, there was 26.33% rate of illiteracy. 61.90% owners responded and 38.10% didn't respond toward ecto-parasitic treatment. 38.43% removed the ticks by hand, 31.02% administrated Ivermectin treatment for mange, 16.20% applied carosine oil for ticks and 14.35% owners used shampoo for lice.

#### OCCURRENCE OF DACTYLOGYROSIS IN SILVER CARP, *HYPOPHTHALMICHTHYS MOLITRIX* REARED IN EARTHEN POND

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The aim of present study was to investigate parasitic infection dactylogyrosis caused by *Dactylogyrus* sp. in silver carp, (*Hypophthalmichthys molitrix*). A total of 146 fishes were studied from July 2014 to June 2015 on monthly basis. Dactylogyrosis, gill infection caused by *Dactylogyrus* sp. was observed in 103 fishes (70.54%). In December maximum numbers (132) of parasites were observed on one fish. The maximum prevalence and mean intensity (100%, 82) was observed in December. A seasonal pattern of occurrence of diseases on gills of *H. molitrix* was observed. Infection level was low in summer as indicated by lower prevalence and mean intensity (50%, 5.3) respectively. In autumn prevalence and mean intensity increased (86.36%, 44.06) as compared to summer. During winter prevalence dropped to 79.94% but the mean intensity attained its maximum value (76.0). However, during spring prevalence and mean intensity both dropped to 61% and 11.9 respectively. These values further dropped in June (prevalence 40% and mean intensity 7). Negative binomial distribution of parasites was observed in all months. Histological observations of infected gills showed severe pathological changes such as: loss of primary and secondary lamellae, cellular atrophy of primary lamellae, hyperplasia of tissues, shrinkage of blood vessels and necrosis of primary and secondary lamellae. *Dactylogyrus* sp. attack on gills was more pathogenic to silver carp during winter than the rest of the year. Epidemiology and gill pathology due to dactylogyrosis in silver carp is discussed.

**ANTI-LEISHMANIAL ACTIVITY OF ALKALOIDS ISOLATED FROM *BERBERIS GLAUCOCARPA* AGAINST PROMASTIGOTES OF *LEISHMANIA TROPICA* CLINICAL FIELD ISOLATE KWH<sub>23</sub>**

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Seven ethanol extracted alkaloid compounds viz. Berberine, 8-Trichloromethyl dihydroberberine, Palmatine, Columbamine, 10-Hydroxy chondrofoline, 10-Methoxy chondrofoline and Tetrandine isolated from *Berberis glaucocarpa* were tested *in vitro* for toxicity against promastigotes of *Leishmania tropica* clinical field isolate KWH<sub>23</sub>. Four different concentrations of each compound and one negative control were prepared and were placed in two 96-well microtitre plates containing  $1 \times 10^5$  *Leishmania tropica* Promastigotes/well. The stock compounds' concentration was 400 µg per one mL of Dimethylsulfoxide (DMSO) and was used in 100 µM, 75 µM, 50 µM, and 25 µM aliquots. The plates were incubated at 26°C for 48 hours and the quantity of *Leishmania tropica* promastigotes in each well was demonstrated microscopically by using an Improved Neubauer Haemocytometer. Interestingly three compounds viz, 8-Trichloromethyl dihydroberberine, 10-Methoxy chondrofoline, and Tetrandine eliminated all the *Leishmania tropica* promastigotes, while the remaining 4 compounds also showed promising antileishmanial activity. Fifty percent inhibitory concentration (IC<sub>50</sub>) as evaluated in GraphPad Prism 5 Software was 0.7547 µM, 0.7563 µM, 0.7544 µM, 0.7319 µM, 0.7520 µM, 0.7563 µM, and 0.7563 µM for Berberine, 8-Trichloromethyl dihydroberberine, Palmatine, Columbamine, 10-Hydroxy chondrofoline, 10-Methoxy chondrofoline, and Tetrandine respectively. Moreover, these compounds were also found to be less toxic to THP-1 cells. Our results indicate that these alkaloid compounds could be useful in the development of new anti-leishmanial drugs.

**SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF TOXOPLASMOSIS AMONG PREGNANT WOMEN IN TEHSIL KHWAZA KHELA SWAT**

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Toxoplasmosis is a parasitic zoonotic infection caused by *Toxoplasma gondii* which is an obligate intracellular protozoan parasite. This study aimed to evaluate the Sero-prevalence and associated risk factors of toxoplasmosis among pregnant women in Tehsil Khwaza Khela Swat. In the present study, a total of 100 samples were diagnosed for determining the seroprevalence of toxoplasmosis in which 18% were found positive for Anti-*T. gondii* IgG/IgM antibodies. The maximum cases (28%) were reported in the month of March and minimum cases (5%) in the month of April. The highest prevalence rate (30%) was reported in Shalpin union council while the lowest prevalence rate (0%) in Fatehpur union council. The infection was more prevalent (28%) in pregnant women having age group of 36 years and above, while the prevalence rate was lower in women having age group of 15-25 years. Illiterate pregnant women were having more prevalence rate (25%) and prevalence rate was 0% in graduates and post graduates women. Similarly 20%

cases were reported in pregnant women working in the houses and 0% in women working in private sector or public sector. Based on history of disease in family; 66% cases were reported from those who had history of disease in family, while 33% cases in those having no history of disease. Lower class women and those residing in non-cemented houses were having 20% rate of infection while 14% cases were from the upper class women and 15% from those who lived in cemented houses. The infection prevalence rate was 19% in those people keeping domestic animals and was 15% in those having no domestic animals. The prevalence rate was higher (55%) in those women having previous infection, while it was lower (44%) in those having no previous infection. Muscles aches were accounting for 33%, influenza 22%, confusion 16%, headache 16%, while 11% pregnant women were found to have no signs and symptoms. The present study concluded that various risk factors have influence on the transmission of toxoplasmosis such as ingestion of raw or dirty vegetables or gardening, eating of raw or undercooked meat, contact with cattle, cats for recreational purposes. The infection can be prevented by taking control measures like avoiding contact with domestic animals and cats, avoiding use of raw meat and vegetables, cleanliness of dwelling places and awareness campaigns about the risk factors of infection.

#### **EFFECT OF VARIABLE HUMIDITY LEVELS ON OVIPOSITION OF *HYALOMMA* TICKS**

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The effects of different levels of relative humidity (RHs) i.e. 30%, 60% and 90% were tested on various reproductive parameters of *Hyalomma anatolicum*, an ixodid tick that acts as vector for Piroplasmosis in cattle, buffalo sheep and goat. It was found that the oviposition increases with increase in relative humidity. The number of eggs oviposited was affected significantly by the effect of RH. Fewer eggs were laid by ticks at 30% RH regime compared to those in 60% and 90% RH. The percentage viable eggs was found to have associated in such a manner that, at RH 60%, More viable eggs were oviposited and the percentage of hatched larvae was found associated with 60% relative humidity. The reproductive fitness index values were highest in females held at 95% RH group, although significantly more larvae hatched at 60% RH. The ideal reproductive conditions for *Hyalomma anatolicum* under laboratory conditions appeared 60% RH. While mating occurred at all humidity levels, none of the females laid eggs at RH 30. Most female ticks oviposited after 20 days when moved to 60 % RH.

#### **PREVALENCE AND ASSOCIATED FACTORS OF HEAD LICE INFESTATION AMONG PRIMARY SCHOOL CHILDREN OF TEHSIL KHWAZA KHELA SWAT**

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The present study was conducted to evaluate the prevalence of head lice infestation in primary school children of Tehsil Khwaza Khela Swat, from March 2015 to August 2015. The data

of this study was analyzed and processed in correlation with locality, age group, class, socioeconomic status, hair color, hair length, dandruff, lubrication, bed sharing, etc. of the participant children. In this study, out of total 200 children, 114 cases were observed as positive for head lice infestation which shows a prevalence rate of 57%. The influence of age on infestation rate was very prominent in our study, as the pupils of the age group 5-7 years were found to have significantly higher rate of infestation (66%) compared to older ones of the age group 11-14 years (44%). The impact of socioeconomic status upon the infestation rate was also detected in our study, as the infestation was found higher in individuals of low socioeconomic status. The prevalence rate was found higher in lower class families (69%) compared to upper class (0%). Pupils having lengthy hair were found to be more susceptible to the infestation (63%) than those with short and long hair. Similarly pupils with black hair were found to have more risk of infestation (59%) than those with lighter hair color. The prevalence of infestation in pupils having black hair was 59%, followed by brown (52%) and yellowish (50%). The prevalence of this infestation was found higher (65%) in students who were having dandruff in their hairs and was also higher (62%) in pupils with using hair oils. The infestation of head lice was also found to be higher in individuals sleeping alone which is 60% compared to that of shared individuals (56%).

#### **SERO-PREVALENCE OF BRUCELLOSIS AND ASSOCIATED RISK FACTORS IN PREGNANT WOMEN OF TEHSIL MATTA SWAT**

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In this study, a total of 100 blood samples were collected from pregnant women of Tehsil Matta Swat to determine the sero-prevalence of brucellosis in the area. The blood samples were tested by serum agglutination test for the presence of anti- *Brucella* antibodies. Out of 100 samples, 28% cases showed positive results. The month-wise high prevalence of brucellosis (54%) was reported in the month of May while low prevalence (30%) was reported in the month of March. Based on union councils, the high prevalence rate of brucellosis (44%) was determined in union council Beha. Age-wise high prevalence rate (33%) was reported in the age-group of above 36 years while the low prevalence rate was reported in the age-group of 15-25 years. In the present study, the higher prevalence rate was reported as 32% in illiterate females while minimum prevalence 0% was reported in higher secondary, graduate and post graduate females. Based on employment status, the highest prevalence rate was reported in house wives (29%) whereas the lowest prevalence rate (14%) was reported in public service females. Similarly high prevalence of 33% was reported in those females in whose family the infection was present while the low prevalence rate (25%) was reported in those individuals whose family members were having no infection in the past. Similarly the highest prevalence rate of 37% was found in females of lower class, whereas the minimum prevalence rate of 17% was found in females of higher class. In this survey, the higher prevalence (40%) was reported in females residing in non-cemented houses while low prevalence (18%) was reported in females of cemented house. The highest prevalence rate of 30% was found in those people having domesticated animals while lowest rate (22%) was reported in those people who have no domesticated animals. On the basis of previous infection, higher prevalence rate of brucellosis (64%) was observed in pregnant females already infected with brucellosis while lower prevalence rate (35%) was in those pregnant female having no previous

infection. In this study, the highest prevalence rate (50%) was reported in those pregnant women who were having low range of infection (1:40 +) while the lowest prevalence rate (7%) was reported in those pregnant females who were having high range of infection (1: 320 +). Maximum infected women (39%) were having joint and muscle pain whereas only 7% were reported to have hypo-gastric pain.

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**DIAGNOSTIC STUDY OF *TOXOPLASMA GONDII* IN FREE-RANGE (FR) DOMESTIC CHICKENS IN TEHSIL KABAL SWAT**

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*Toxoplasma gondii* is one of the intracellular protozoan parasites which is mostly prevalent in human and animals, including poultry in which chickens are considered an important intermediate hosts of *T. gondii*. The prevalence of *Toxoplasma gondii* in free range chickens is a good indicator of the prevalence of *T. gondii* oocysts in the environment because chickens feed from the ground. This study was conducted from March 2015 to August 2015 to determine the seroprevalence of toxoplasmosis in domestic chickens of Tehsil Kabal Swat. The seroprevalence of *Toxoplasma gondii* among 120 chickens (*Gallus domesticus*) of different areas of Tehsil Kabal Swat were assessed in which 15 positive cases occurred that showed a prevalence rate of 12.5% in the area. The prevalence was higher in female (13%) than male (13%). Based on age, the highest prevalence (13.33%) was observed in chickens of 2 years. Union Council Qalagy were having highest prevalence rate i.e. 25%, similarly the higher prevalence was reported in chickens consuming normal food and highest prevalence occur in poor condition chickens which is 93.33%. It is concluded that chickens play an important role in transmitting the *T. gondii* infection in humans and other animals, so it is necessary to take preventive measures for controlling the infection.

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**SECTION - V****FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY,  
MARINE BIOLOGY****1. ECOLOGY AND ENVIRONMENTAL POLLUTION****WATER QUALITY OF AMPHIBIAN HABITATS IN SINDH, PAKISTAN**

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Amphibians lay their eggs into water, where they remain confined till adulthood; therefore quality of their primary habitat has great impact on their growth, development as well as survival. For this perspective, amphibian habitats in a main subdivision of District Jamshoro "Sehwan" was investigated for the novel and detailed record of amphibian environmental status. Field surveys and water analysis was conducted for three years (2011-2013) from March to October at six amphibian habitats. Analytical instruments and methodology was applied during analysis of various physico-chemical parameters including pH, EC (electric conductivity), TDS (total dissolved solids), T-Hard (total hardness), T-Alk (total alkalinity), Cl (chloride), SO<sub>4</sub> (sulphate), PO<sub>4</sub> (phosphate), NO<sub>2</sub> (nitrite), NO<sub>3</sub> (nitrate), CO<sub>2</sub> (carbon dioxide) and K (potassium). The value of parameters was recorded as followed: pH (8.2±0.6), EC (2313.9±1157.9), TDS (1507.7±707.8), T-Hard (487.0±267.3), T-Alk (349.2±92.3), Cl (355.3±98.2), SO<sub>4</sub> (467.8±159.2), PO<sub>4</sub> (361.8±86.6), NO<sub>2</sub> (5.3±3.5), NO<sub>3</sub> (6.9±2.7), CO<sub>2</sub> (17.4±3.4) and K (75.5±10.3). Value of each parameter was compared with the optimal level criteria of EPA (Environmental Protection Agency) and other scientific literature to identify the condition of amphibian ponds. The results revealed highly contaminated status of ponds as value of all parameters (except pH and CO<sub>2</sub>) was high. It was also recorded that the water quality of all amphibian ponds was different from each other and value of parameters changed monthly as well as yearly. Highest rate of contaminants was recorded during year-2012, while least value of evaluated parameters was recorded in year-2013. This persistently unsuitable environment may affect amphibian population negatively.

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**WATER CONTAMINATION MAY THREATEN AMPHIBIAN POPULATION IN  
DISTRICT JAMSHORO, SINDH-PAKISTAN**

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Amphibians are threatened by a number of factors including environmental contamination which has adverse effects on them; hence present study was carried out at amphibian ponds situated in Taluka Kotri and Taluka Thano Bula Khan of District Jamshoro. The physico-chemical study was carried out from March to October for three years *i.e.* 2011 to 2013 using instrumentation and methodology of analytical grade. The value of electric conductivity (EC) was recorded by the



measuring unit of  $\mu\text{S cm}^{-1}$ , whereas  $\text{mg L}^{-1}$  was the measuring unit of all other parameters such as total dissolved solids (TDS), total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl), Sulphate ( $\text{SO}_4$ ), phosphate ( $\text{PO}_4$ ), nitrite ( $\text{NO}_2$ ), nitrate ( $\text{NO}_3$ ), potassium (K) and carbon dioxide ( $\text{CO}_2$ ). Present investigation revealed unsuitable water quality of six study sites of Taluka Kotri where value of EC ( $2280.4 \pm 734.6$ ), TDS ( $1557.7 \pm 501.0$ ), T-Hard ( $361.6 \pm 70.8$ ), T-Alk ( $310.1 \pm 50.6$ ), Cl ( $320.5 \pm 58.9$ ),  $\text{SO}_4$  ( $394.2 \pm 87.0$ ),  $\text{PO}_4$  ( $395.2 \pm 103.4$ ),  $\text{NO}_2$  ( $3.6 \pm 1.2$ ),  $\text{NO}_3$  ( $6.0 \pm 2.7$ ) and K ( $70.3 \pm 8.2$ ) was extremely high up to dreadful level, although value of pH ( $8.0 \pm 0.6$ ) and  $\text{CO}_2$  ( $18.7 \pm 3.7$ ) was normal. During the water quality analysis of other six amphibian habitats in Taluka Thano Bula Khan, it was recorded that pH and  $\text{CO}_2$  were the only parameters whose values ( $8.0 \pm 0.6$  and  $18.2 \pm 3.32$  respectively) were normal in whole study area, though value of other parameters including EC ( $2821.8 \pm 1202.2$ ), TDS ( $1861.8 \pm 759.0$ ), T-Hard ( $367.7 \pm 56.0$ ), T-Alk ( $351.7 \pm 54.9$ ), Cl ( $377.6 \pm 135.4$ ),  $\text{SO}_4$  ( $463.8 \pm 125.5$ ),  $\text{PO}_4$  ( $439.2 \pm 124.9$ ) and K ( $67.5 \pm 10.7$ ) exceeded above the normal limit. It was also determined that the concentration of  $\text{NO}_3$  ( $2.2 \pm 1.6$ ) and  $\text{NO}_2$  ( $6.1 \pm 4.3$ ) varied from low to high value, but remained unsuitable for most of the time. Present research recorded high rate of pollution in both amphibian environments, where conservation actions are urgently required for the conservation of these delicate animals.

#### STUDIES ON HEAVY METAL TOXICITY AND WATER QUALITY PARAMETERS OF RIVER SUTLEJ, PUNJAB, PAKISTAN

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This six month study extending from July through December, 2013 was conducted in district Kasur, Punjab, Pakistan. Water samples were collected from tributaries *i.e.* Bias, Rohi Nallah and Kasur city drain and river Sutlej. pH of the water ranged between 6.88-7.29, dissolved oxygen 2.23-3.87 mg/l, electrical conductivity 1.0-1.9 ms/cm, salinity 0.4-0.8 ppt, total dissolved solids 654-1054 mg/l, temperature 25-26 °C, chlorides 110-500 mg/l, total alkalinity 120-460 mg/l. The concentrations of Mn and Ni in water were below the detection limit. The metal concentrations were recorded highest from Kasur city drain while lowest metals' concentrations were recorded from Bias tributary.

#### DISTRIBUTION AND DIVERSITY OF AMPHIBIANS & REPTILES AT KALABAGH, DISTRICT MIANWALI, PAKISTAN

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The study is a part of M Phil research conducted during March and September 2015 at Kalabagh Game Reserve which is located at N 32.52° and E 71.39° in District Mianwali. The study area is a part of the Salt Range in Punjab, Pakistan. A thorough review of the available literature revealed that some parts of the Kalabagh Game Reserve were herpetologically unexplored. Therefore, study was planned and conducted to record the diversity and distribution of amphibians

and reptiles from around 21 km<sup>2</sup> area in the Kalabagh Game Reserve being managed by The Nawab of Kalabagh. Twelve 12 different locations in the study area were search for herpetological elements through four visits each of 7-10 days duration. Prior to the field visits, a thorough review of the available literature was done to have an idea about the existing species in the study area. The study area represents different types of habitats and terrains including cultivated lands, wild lands, semi desert area, stony planes and Rocky Mountains. Similarly, some species are nocturnal in feeding habits whereas others are diurnal; therefore, different direct and indirect methods were applied to study various groups of the herps. Field visits were carried out between 9:00 am to 4:00 pm for diurnal species and for two hours after dusk for the nocturnal species. Most of the species were identified at the spot almost all the species encountered were collected and preserved for detailed studies. Field notes and observations on habitats were made, GPS coordinates were recorded and still photographs of different species were taken as the evidences of their existence. Total 21 species belonging to three orders, 13 families and 18 genera were recorded including six amphibians and 15 reptiles. Amphibians included two toads and four frogs whereas; reptiles included one turtle, nine lizard and five snake species. Three species out of the five recorded snakes are poisonous rests all the amphibian and reptiles are non-poisonous. Most of the recorded species were observed directly whereas indirect evidences of some of the species were also observed like tracks, burrows, molts and interviews with local residents that confirmed the existence of the species in the area. One species of snakes; Slender blind snake, is recorded for the first time from this area. Out of the recorded 21 species, three were found among endemic species to Pakistan including two lizards; Agrore valley agama and Rohtas gecko and one snake.

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#### HABITAT CHANGING VERSUS FISH LANDING DECLINE IN KARACHI SEA AREA

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Decline in the marine fish land in Pakistan is reasonably is increase into the recent years. Different reasons are having different weight age into the declining index. These authors observed a new reason for decline in fish landing. It is named as "Changing into the habitat". It is found that there are five reasons for changing habitat Karachi. a) Rahray coast slaughter house waste is increased. B) Clifton coast, park Ib n Qasim encroach over 1000 feet sea shore area, c) Shreen Jinnah Colony extension of Karachi Harbor, d) Mai kolachi cutting of 2000 well grown mangrove trees and encroach over its land, d) Manora increase of channel length for large vessels entry into the harbor affect the habitats up to Pacha Bundar, e) Malir and Layri River waste heavily change the habitat of back water and mangroves. Large number of spices completely eradicated. Where construction of new Kanap at "Kap mount" is not yet disturbed the habitat. In this study it observed that at the "eastern coast" of Karachi deep water converted into shallow water and in the western coast the shallow water is become deep water, due to above noted reasons. In support of this hypothesis the author is coating few examples. First, die of bivalve shell at Clifton (eastern beach) since few years is due to reason that the deep water becomes shallow. The "Western coast" water is become deep water, because the fauna and flower of deep seawater are largely grown at Manora-Paradise Point Area. Even the sea sand colour of Kaka and Manora is become same like the color of Mubarak village sand. We know Mubarak village is a deep sea area.

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### SEASONAL VARIATION OF SOAN RIVER USING INSECTS AS WATER QUALITY INDICATOR

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In the present study, the effect of pollutants produced by human dwelling, agricultural and industrial activities on aquatic invertebrate's communities of Soan River, Pakistan was studied. Four different sites were selected on the basis of variation in microhabitat accessibility to examine the pollution in water. Samples were collected from these sites during October, 2014 and April, 2015. From all sites physical conditions, water samples and macroinvertebrates were collected. T.D.S (Total dissolved solids), nitrates were high at two sites (downstream of the river) in both seasons while sodium and cadmium contents were high at all sites and other studied parameters (pH, conductivity, chloride, sulfate, nitrite, calcium, total hardness, sodium, potassium, magnesium, chromium, aluminium, copper, dissolved oxygen (D.O), nitrogen and phosphorous) were according to WHO standards. A total 620 individuals of aquatic insects were collected during the studied sites which belong to 8 orders and 17 families of invertebrates. Gerridae, Hydrophilidae, Gyrinidae, Culicidae and Chironomidae were the most abundant in month of October while Gerridae, Elmidae and Hydrophilidae were in April. Total abundance was used to estimate the quality of water of the sites. Most of the biotic index showed that water was in good quality on upstream stations rather than downstream station in April, but in October upstream station were polluted too. This study showed that aquatic insects could provide useful bioindicatorsto approach the biomonitoring in relation to water physio-chemical parameters to classify, compare and assess the quality of water of freshwater streams in Pakistan.

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### STUDIES ON HEAVY METAL TOXICITY AND WATER QUALITY PARAMETERS OF RIVER SUTLEJ, PUNJAB, PAKISTAN

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## 2. FRESHWATER BIOLOGY AND FISHERIES

### REGRESSION STUDIES OF PLANKTONIC PRODUCTIVITY AND INCREASE IN FISH BIOMASS ON PHYSICO-CHEMISTRY OF PONDS STOCKED WITH METALS MIXTURE STRESSED FISH

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The 90-day five fish species viz. *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cirrhina mrigala*, *Labeo rohita* and *Catla catla* were exposed to sub-lethal concentrations of metals mixture (Zn+Pb+Mn) and metal free media (control) in glass aquaria for twelve weeks under constant laboratory conditions. After laboratory exposures, these groups (metals mixture stressed and control) were stocked separately in earthen ponds and reared for twenty four weeks under semi-intensive culture system. Three replications were used for each group. Weekly data on dry weights of planktonic biomass, increase in fish biomass and physico-chemistry of pond's water viz. pH, temperature, dissolved oxygen, phosphates, nitrates, sodium, potassium, total ammonia, total hardness, total alkalinity, electrical conductivity, light penetration and chlorides were collected and analyzed statistically. Correlation and regression analyses were performed to find-out statistical relationships among various parameters defined for this study. It was concluded that various physico-chemical parameters of water exerted significant impact on the planktonic productivity and increase in fish biomass of both metals mixture (Zn+Pb+Mn) stressed and control fish ponds. Among the variables, temperature appeared as a variable that exerted significant impact on the planktonic productivity of stressed as well as control ponds. In both stressed and control ponds, light penetration exhibited positive and highly significant ( $p < 0.01$ ) while total ammonia and temperature showed negatively significant ( $p < 0.01$ ) regression on an increase in fish biomass. In control fish pond, dissolved oxygen exhibited negative but significant ( $p < 0.01$ ) regression on an increase in fish biomass.

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### DETERMINATION OF MICRONUCLEI FREQUENCIES AND OTHER NUCLEAR ABNORMALITIES IN PERIPHERAL BLOOD ERYTHROCYTES OF FRESH WATER FISH, *CATLA CATLA* EXPOSED TO BIFENTHRIN

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Bifenthrin (Pyrethroids) belongs to the most commonly used pesticides throughout the world. Their extensive usage is a threat to the natural environments including aquatic ecosystems. Although bifenthrin are rapidly degraded in soil and plants, but they are extremely toxic to the fish. Keeping in view the high sensitivity of fish to bifenthrin, the present study was conducted to detect the genotoxic effects of waterborne bifenthrin in peripheral blood erythrocytes of freshwater fish, *Catla catla* using micronucleus test. On the basis of 96-hr LC<sub>50</sub>, fish were exposed to four sub-

lethal concentrations of bifenthrin (10, 20, 33 and 50 % of LC<sub>50</sub>) with negative control, separately, to investigate the micronuclei frequencies and other nuclear abnormalities for 30 days. The exposure of waterborne bifenthrin to *Catla catla* resulted into significantly higher mean micronuclei frequency due to 50% LC<sub>50</sub> while negative control showed significantly lower mean frequency. The total frequency of other nuclear abnormalities, designated as binucleated, dumb, blebbed, notched and deshape nuclei, were significantly higher due to 50% LC<sub>50</sub> exposure, followed by that of 33%, 20%, 10% LC<sub>50</sub> and negative control. The differences among all the exposure treatments, regarding total frequency of other nuclear abnormalities, were significantly ( $p < 0.05$ ) different. This study also revealed micronucleus frequency indices as the sensitive, rapid and useful methods in determining the potential genotoxicity of bifenthrin in fish.

#### AN ASSESSMENT OF GROWTH CONDITION OF SPOTTED SCAT, *SCATOPHAGUS ARGUS* (LINNAEUS, 1766) FROM PAKISTAN COAST

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In this research, condition factor (K) and relative condition factor (Kn) of a spotted scat, *Scatophagus argus* were investigated in order to confirm the hypothesis that heavier fishes are in good condition than lighter fishes. Approximately 54 specimens of different sizes were collected from the commercial landings at fish harbours of West wharf and Korangi creek, Pakistan. Fresh samples of *S. argus* were analyzed in the laboratory. Total length of Fish in centimeter (cm) and weight of fish in grams (g) were measured with the help of measuring board and by an electronic balance, respectively. Total length and weight of fish were investigated ranging from 10.0-20.3cm and 29.0-199.0g, respectively. The results for the condition factor (K) were obtained between 2.08 to 3.11 while, results for the relative condition factor (Kn) were obtained between 0.81 to 1.86 hence, shows that *S. argus* is in good condition at Pakistan coast and the environmental factors of Pakistan coast are favorable for the healthy growth of *S. argus*.

#### CURRENT STATUS OF MAHSEER IN RIVER SWAT KHYBER PAKHTUNKHWA

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Fishes are considered as important indicators for exploring the status of aquatic environment because they inhabit different types of aquatic habitats. Among many fish species, Mahseers are of great economic importance due to their delicious taste. But currently their population in open water body is declining due to several anthropogenic pressures such as habitat loss and illegal hunting. Therefore the present study was focused on the diversity and distribution of Mahseer (*Tor* genus) in river Swat during May to August 2015. Collection was made from different sites of river Swat such as, Fizagat, Bypass, Nawaykalay, Ingaroderai, Rahim abad, Qambar, Odigram, Balogram, Gogdara, Manyar, Ghalegay, Barikot, Landakay, Shamoza, Jalala, Thana, Chakdara, Totakan and Bosak through Mesh net, Cast net, Fishing rod and Hooks. The collected samples were identified by following various taxonomic keys and were then preserved in 10% formalin. We reported two

species of *Tor* genus which were; *Tor neilli* and *Tor mussullah*. In the current study, *Tor neilli* was reported for the first time from river Swat which has not been reported in the previous literature. Beside Mahseer species, other fish species like *Schizothorax plagiotomus*, *Labeo rohita*, *Cyprinus carpio*, *Mustacembalus armatus*, *Glyptothorax cavia*, *Hypophthalmichthys molitrix* and *Channa marulius* were also reported. The less diversity of Mahseer in river Swat might be due to changes in the physico-chemical parameters of water which either cause fish mortality or migration. Therefore the conservation of aquatic habitat is necessary for maintaining a rich ichthyofauna in wild.

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**SYNERGISTIC EFFECT OF *GEOTRICHUM CANDIDUM* AND VITAMIN C ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF *LABEO ROHITA* (HAM.)**

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Vitamin C and probiotics both have a positive impact on growth and immune response of fish. In order to evaluate the combine effect of L-ascorbyl-2-polyphosphate (APP) and locally isolated probiotic *Geotrichum candidum* QAUGC01, a 90 days feeding trial was designed. Experiment was conducted in replicate of three by using completely randomized design. Advanced fry of *Labeo rohita*, average body weight  $3.52 \pm 0.073$  g were evenly distributed in four groups. The control group was fed basal diet while three treatment groups were reared on basal diets supplemented with 150 APP kg<sup>-1</sup> diet, probiotic QAU GC-01 10<sup>12</sup> CFU kg<sup>-1</sup> diet and mixture of probiotic + APP. Results indicated that dietary supplementation of APP and probiotic significantly improved the growth rate (% weight gain, specific growth rate) feed conversion ratio, activity of intestinal enzymes (protease, amylase) and immune response (lysozyme activity, WBCs count and immunoglobulin (IgM) level of fish. However, the mixture of probiotic + APP showed more significant effects on growth performance and immune response as compared to supplement them alone. Based on the results, it appears that the vitamin C and probiotic *G. candidum* synergize the effects of each other. Thus, suggest a new approach for improving fish production

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**EVALUATION OF NANOPARTICULATE DIET FOR REARING OF ADVANCED FRY OF *HYPOPHTHALMICHTHYES MOLITRIX***

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In the present study, 35% Nanoparticulate diet having particle sizes ranging between 40 - 80 nm was prepared and compared with microparticulate diet (particle size 23-85 µm) for feeding advanced fry of *Hypophthalmichthys molitrix*. Fry average body weight  $3.0 \pm 0.03$  g were evenly distributed into three groups (25 fry per fiberglass tank) following a completely randomized design. The experiment was conducted in replicate of three. The first group of fry was fed crumbled microdiet while the other two groups were offered nanodiet either in crumbled or powdered form. After 90 days of feeding, significant ( $P < 0.05$ ) effect of different form of prepared diets on the

weight gain % and specific growth rate was observed. The highest growth rate was observed in a group of fish fed powdered nanodiet followed by crumbled nanodiet. However, the lowest weight gain and specific growth rate were evident in a group of fry fed crumbled microdiet. The intestinal protease and amylase activity followed the same trend, highest with a powdered nanodiet, while lowest values were observed in a group of fry fed crumbled microdiet. Based on the results, it appears that the efficiency of feed can be improved by converting it in nanoform, but further research on leaching of nutrients in water is required before its commercial use in aquaculture.

**HISTOLOGICAL PATTERN OF THE REPRODUCTIVE STATE OF THE RECRUDESCENT OVARY OF *LABEO ROHITA* OF KETIBANDAR THATTA & AL-MANZAR JAMSHORO, SINDH**

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*Labeo rohita*, a commercial carp was collected from the different inland waters. The ovaries were isolated from each fish and processed for the purpose of histological studies. Ovarian structure during this period indicates that all the follicles except few were in the primary or secondary oocyte stage. This condition of fish shows that the ovaries are progressing uniformly which is the sign of fish spawning once in a year.

**RELATIVE DIVERSITY OF SNAKE-HEADED FISHES FROM ANTHROPOGENICALLY IMPACTED STRETCH OF THE RAVI, PAKISTAN**

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The study was conducted for consecutive two years (2014-2015) to assess the relative diversity of common snake-headed fish fauna of the Ravi. The fishes were sampled from six sites (1 = Shahdara, 2 = Thokar Niaz Beg, 3 = Mohlanwal, 4 = Sundar, 5 = Manga Mandi, 6 = Balloki Headworks) of anthropogenically affected segment of the Ravi flowing along the Jhok Reserve Forest. The diversity, evenness and richness of four species of snake-headed fishes i.e., *Channa marulius*, *Channa striata*, *Channa punctata* and *Channa gachua* were calculated. Statistical analysis revealed that diversity indices were decreased gradually. In addition, stressed populations of *Channa marulius* and *Channa striata* were observed among the rich populations of *Channa punctata* and *Channa gachua*. The study necessitates the implication of proper conservatory measures and therefore, suggests future studies on pollution impacts and breeding biology of the said stressed fish species of the Ravi.

**ROLE OF PHYTASE SUPPLEMENTATION IN IMPROVING NUTRIENT AND MINERAL DIGESTIBILITY OF *CIRRHINUS MRIGALA* FINGERLINGS FED ON PLANT BASED DIET**

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Presence of phytic acid in plant ingredients reduces the bioavailability of protein and minerals to fish ultimately resulting in reduced fish growth. The present research work was conducted to examine the effects of phytase supplementation (0, 500, 1000, 1500 and 2000 FTU kg<sup>-1</sup>) to soybean meal diet on nutrients and mineral availability for *Cirrhinus mrigala* fingerlings. Chromic oxide was added as indigestible marker. *C. mrigala* fingerlings were stocked in V-shaped tanks in Fish Nutrition Lab particularly designed for the collection of feces. Triplicate tanks were used for all treatments. Feed was given to fingerlings at the rate of 5 % of live wet weight of fish. From each tank feces were collected twice daily. Collected feces were dried at 60°C and pooled for each treatment. Water quality parameters viz., DO, temperature and pH in each tank were monitored throughout the study period. The results showed that 1000 FTU kg<sup>-1</sup> level in soybean meal based test diets increased the bioavailability of nutrients and minerals to *C. mrigala* fingerlings which in turn resulted significant (P<0.05) increase in growth performance parameters. Therefore, phytase supplementation at 1000 FTU kg<sup>-1</sup> level in plant ingredient (soybean meal) based diets was suggested as optimum level of phytase supplementation to enhance the release of chelated nutrients and minerals. Use of phytase in diets reduces the nutrient and minerals discharge into aquatic environment resulting in less aquatic pollution. Supplementation of phytase also helped in developing cost-effective and eco-friendly fish feed.

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**USE OF DNA BARCODING TO CONTROL THE ILLEGAL WILDLIFE TRADE: A CITES CASE REPORT FROM PAKISTAN**

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Illegal wildlife trade is a great threat to the conservation efforts made worldwide to save wildlife species and their parts. Use of molecular methods, including DNA barcoding, is gaining acceptance to detect cross-border movement of endangered species. Here we report the utility of DNA barcoding in the detection of smuggling of an endangered turtle species from Pakistan. The consignment labeled as “fish meat” was intercepted at a Pakistani port and was tested for its source using DNA Barcoding with fish-specific primers. Sequences from the samples from this consignment matched (99%) with those from *Lissemys punctata* (Indian flap-shelled turtle), a species listed by the Convention on International Trade in Endangered Species (CITES). This report highlights the problem of smuggling protected species under false pretenses and the importance of DNA barcoding in stopping such illegal trade.

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### REGRESSION STUDIES OF PLANKTONIC BIOMASS ON PHYSICO-CHEMICAL PARAMETERS OF POULTRY MANURE FERTILIZED POND

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The regression equations of dry weight of planktonic biomass upon physico-chemical characteristics of one acre pond fertilized by poultry droppings was obtained after six months of experimental period by using stepwise regression method. Pond was stocked with 2000 fingerlings of five fish species viz. *Labeo rohita*, *Catla catla*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* and fertilized with poultry droppings at the rate of 0.17 g nitrogen per 100g fish weight on daily basis. During this study period, the physico-chemical variables viz. pH, electrical conductivity, dissolved oxygen, temperature, total alkalinity, carbonates, bicarbonates, total hardness, calcium, magnesium, ammonia, nitrates, phosphates, chlorides, sodium, potassium and dry weight of planktonic biomass was monitored on fortnightly basis. The regression of pH on dry weight of planktonic biomass held it responsible for more than 83.02% of variation in biomass. Magnesium along with pH, potassium, carbonate, ammonia, dissolved oxygen and chloride exhibited negative but significant regression on increase in planktonic biomass with  $R^2$  of 93.98. Other variables also showed some contribution towards variation in biomass under the treatment in these regression studies.

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### PROBLEMS FACED BY PAKISTANI SEAWATER AQUARIUM FISH HOBBYISTS AND ECONOMIC SOLUTIONS THEREOF

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There are about 800 seawater aquarium fish's hobbyists and 12 aquarium fish suppliers/shopkeepers who are meeting their demands. About 30,00,000 freshwater aquariums can be converted into seawater, if the major problems of maintenance are properly addressed. At present 90 % demand of marine aquarium fishes, invertebrates and equipment is being fulfilled by the imported products. Rest of the 10 % demand is being covered by five local suppliers. Mortality rate of marine fishes and invertebrates is significantly high as compared to freshwater fishes. Seven major problems were found to be the most common during a survey of hobbyists *i.e.* cooling, heating, protein skimming, ammonia, phosphate and lightening. To handle these problems, imported costly instruments and medicines are being purchased from the market or imported. In the present study economical solutions by employing local products are explored. A) For a 3000 L aquarium, a chiller plant of Rs. 25,000/- along with daily electricity consumption costing about Rs.100/- per month is being used. Whereas locally produced model reduces the temperature up to 6° C. B) Heating cost is Rs.100 per day by use of automatic electric heater. Whereas locally introduced model costs Rs.1/- per day. Authors improvised this model by adding a steel pot, along with gas burner between aquarium and sump/ filtering unit. C) A protein skimmer for a 3000 L aquarium costs about Rs. 35,000/-. Whereas locally made protein skimmer costs Rs. 500/- only that

is more efficient than the imported one. D) Ammonia controlling medicines cost Rs. 500/- per week. As an alternative firstly, colonies of nitrifying bacteria to convert ammonia into nitrites and nitrites to nitrates are employed; secondly use of living green seaweeds like *Chaetomorpha* sp. is recommended; thirdly use of mangrove plants in the sump/ filtration unit is suggested. E) Phosphate richness is due to leftover food and death and decay of living organisms, which is reduced by using medicine that costs Rs.1500/- per week. To overcome this problem fresh sand and aquarium cleaning crews (animals) are introduced to handle the phosphate level. F) Lightening by the metal halide light costs Rs. 20/- per day. While locally produced sunlight model costs nothing as the sunlight is being reflected into an aquarium by two to three mirrors. The models proposed in the present study are improvised by us and result of several experiments and running marine aquariums of 3500 L for a couple of years economically.

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**FROM PROTEIN RICH PLANT SOURCES AND ITS EFFECTS ON GROWTH,  
NUTRIENT UTILIZATION AND MEAT QUALITY OF YELLOW FIN SEA BREAM  
(*ACANTHOPAGRUS LATUS*)**

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Feed formulation [or fish in terms of its palatability and energy provision has been a subject of recent research in the field of aquaculture. The consumption of fish meal in fish diets is high, mostly owing to their outstanding nutritive characteristics. Though, In order to reduce major reliance on these supplies in feeds, use of sustainable alternatives is important. Therefore, current research was planned to develop high energetic diet by replacing fish meal with protein rich plant sources (vegetable by-products) on the daily growth performance, utilization of feed nutrients, flesh quality in terms of fatty acid conformation in commercially important yellow fin sea bream (*Acanthopagrus latus*). During the preparation of experimental feeds, fish meal was replaced by corn gluten meal (CGM), soybean meal (SBM) and mustard oil cake (MOC). Four isoenergetic diets (21.4 kg) were manufactured comprising fish meal, soya bean meal, corn gluten meal, mustard oil cake, blood meal, shrimp meal, tapioca Dour, soya lecithin, coconut seed meal, rice bran, APC, fish oil, vitamins and minerals. The diets were tested on the fish juveniles with two replications. The healthy juveniles of *A. latus* (initial body weight 8.5 g and initial body length 3.4 cm) were collected from wild and stocked in glass aquaria (5 fish per aquarium). Each aquarium was supplied with 100 liters filtered seawater and was continuously aerated. The salinity of aquarium water was maintained at 50‰. Water quality parameters were monitored daily. Half amount of the water was replenished daily. Each aquarium was siphoned every day early in the morning. Fishes were fed three times a day with ration of 2% live body weight for 50 days. Results of this research revealed that no harmful effects were observed on the growth parameters as well as feed conversion in yellow fin sea bream as fish meal was substituted by CGM, SBM and MOC at different concentrations. The diets in which fish meal was replaced by SBM showed significantly high growth and nutrient utilization. No significant changes were observed in chemical constituents like protein, carbohydrates, lipids and ash of the whole body of fish among different dietary managements. However, fish meal substitution by soybean meal affected the composition of fatty acids in the diets and tissues. As a result of this change, significant variations in the composition of fatty acids were noted in muscle and liver. In conclusion, 18% fish meal can be replaced by soybean meal in diets of yellow fin sea bream.

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### **THERMO TOLERANCE OF MARINE ORNAMENTAL FISHES AND ECONOMICAL MEASURE TO CONTROL TEMPERATURE LEVEL**

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Cooling and heating of marine ornamental aquarium fishes are very important phenomena. Most of the hobbyists are not successful to control it. Temperatures beyond the tolerance levels of cause mortality and hobbyist compelled to switch over to the freshwater aquarium fishes. In this study the heat tolerance level of 10 species of marine aquarium fishes and 10 species of invertebrate was observed. Temperature was increased by the help of thermostatic heater and cooling by common chiller made for aquarium fish industry. Mortality rate due to change in temperature both in experimental and control sets is observed and plotted graphically. Average temperature tolerance level was observed between 16° to 28° C. An innovative gas heater that was not in practice in the industry all over the world was introduced. Another improvisation was to add steel pot with gas burner between aquarium and filtration unit/ sump. Resultantly up to 6° C cooling was observed *i.e.* by reducing the height of aquarium and increasing numbers of CPU fans at a distance of 6" from the water surface. Such an economical system can prevent marine hobbyists to switch over and boost their earning capacity.

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### **CARCASS QUALITY OF ROHU (*LABEO ROHITA*) FINGERLINGS FED VITAMIN E SUPPLEMENTED DIET**

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The present project was designed to determine the carcass quality of rohu (*Labeo rohita*) fingerlings fed vitamin E supplemented diet. Experimental diets were supplemented with six graded levels of vitamin E concentrations *i.e.*, 0, 25, 50, 75, 100 and 125 mg kg<sup>-1</sup>. The fingerlings were fed at the rate of 2% of live wet weight on their prescribed diets daily for two months. Water quality parameters such as dissolved oxygen, pH and temperature of each experimental unit were monitored constant throughout the feeding trial. At the end of feeding trial, five fish from each replicate were sacrificed for the analysis of whole body proximate composition, fatty acid profile, vitamin E content, thiobarbituric acid reactive substances (TBARs) and antioxidant enzymes (superoxide dismutase, catalase and peroxidase) activities. Results were subjected for one way analysis of variance. The results showed that the vitamin E supplementation non-significantly ( $p>0.05$ ) influenced the crude protein, crude fat and moisture contents of body. Highest activities of antioxidant enzymes were observed in the group fed on highest vitamin E supplementation (125 mg/kg). Moreover, the supplementation of vitamin E (125 mg/kg) significantly decreased the

saturated fatty acids and increased the unsaturated fatty acids level in the body of fingerlings. Conclusively, vitamin E supplementation non-significantly affected the proximate composition of *L. rohita*, while significantly improved carcass unsaturated fatty acids by decreasing lipids peroxidation.

#### PROXIMATE COMPOSITION OF MEAT AND INTERNAL ORGANS OF *RITA RITA* (HAMILTON) IN RELATION TO BODY WEIGHT CATEGORIES

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The proximate composition of the meat and some internal organs usually disposed off *i.e.* gills, liver and gut of wild *Rita rita* belonging to three different weight categories *i.e.*  $\leq 350$ g ( $W_1$ ), 351–550g ( $W_2$ ) and 551–750g ( $W_3$ ) were examined in this study.  $W_1$  showed maximum values of percent moisture in all studied body parts of *R. rita* compared to  $W_2$  and  $W_3$  weight categories. Fish belonging to  $W_3$  weight category fish showed higher crude protein and lipid (%) in comparison to  $W_1$  and  $W_2$ . Highest ash (%) was measured in gills of *R. rita* in  $W_3$  weight category as compared to  $W_1$  and  $W_2$ . Total carbohydrates are usually calculated by difference of the entire proximate parameters and it was observed that liver contain maximum carbohydrates (%) compared to other studied body parts in this study.  $W_3$  weight category was found to have significantly ( $p < 0.05$ ) higher nutrients than  $W_2$  and  $W_3$ . It is found that although gills, liver and gut of wild captured *R. rita* is not consumed directly by human beings but they have considerable amount of nutrients. On the basis of present study inferences, it is concluded that the nutrients composition of *R. rita* are within nutritional range required by humans.

#### GENETIC RELATEDNESS OF *OREOCHROMIS NILOTICUS* POPULATIONS IN RIVER JHELM

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Understanding of genetic variation is important for management of wild populations. The successful utilization of molecular markers has been verified for this purpose. The present research work was conducted to assess the populations genetic structure of *Oreochromis niloticus* populations at different sites of River Jhelum. In this study, the populations of *O. niloticus* were examined by using microsatellite DNA markers. Samples of the fish were collected from the River Jhelum. The sampling localities were as follows: Trimu Headworks, Rasul Barrage, Marala Dhand, Mangla Dam and Pind Dadan Khan. Data was collected and used for calculating various

parameters of population structure genetics using PCR method such as population variations, heterozygosity and population differentiation. The template DNA was subjected to PCR amplification by using species specific primers. The products of PCR were checked by polyacrylamide gel electrophoresis. The size of allele was measured with reference to the relative size of the DNA ladder. The average observed heterozygosity of five populations ranged from 0.71 to 0.78. The  $F_{ST}$  values showed slight genetic variations ranging from 0.105 to 0.221. The deviation from *HWE* (Hardy Weinberg equilibrium) was significant. The results showed inbreeding in the populations of *O. niloticus*. Current knowledge about genetic structure of *O. niloticus* populations would be very useful for defining effective fisheries management policy.

### FISH SCALES AS BIOINDICATOR OF WASTE WATER TOXICITY FROM RIVER CHENAB AT CHAK BANDI DRAIN

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This study was meant to use fish scale as a bio-indicator for pollution that is gradually increasing in natural waters through anthropogenic activities along with water quality parameters assessment and detection of selected Heavy metals in water samples collected from River Chenab and Chakbandi drain that gathers domestic sewage wastes and industrial effluents from Faisalabad and drop into it contaminating this freshwater body. All water quality parameters (pH, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), salinity, conductivity, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) phenols and sulphates) and concentrations of selected heavy metal (Cd, Cu, Mn, Pb, and Cr) were found considerably higher than WHO permissible limits capable of causing ill health effects in aquatic organism. Three selected fish species *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were also collected from the study area of the River Chenab to get the fish scales specimens to detect the effect of pollution on them. In this study a conventional approach has been made to study the scale morphology using a light microscopic scale preparation and photography with a digital camera. Fish scales specimens of selected fish were described qualitatively and observed quantitatively. In fish scale study, several distinguishable characteristics were considered such as the scale size, scale type, overall scale shape, the shape of the margin, and the position of the focus, Annuli, circuli and radii appearances, and the type of radii. Current study results disclosed that there were considerable variations in the scale's morphology. The focus showed maximum deformities (76.19%) in *L. rohita* whereas these were minimum (66.67%) in *C. catla* whereas normal focus was observed maximum in *C. catla* (33.33%) and it was minimum (23.81%) in *L. rohita*. In case of annuli maximum deformities (66.67%) in *C. mrigala* whereas these were minimum (47.62%) in *C. catla* whereas normal annuli were observed maximum in *C. catla* (52.38%) and it was minimum (33.33%) in *C. mrigala*. In case of circuli maximum deformities (76.19%) in *C. mrigala* whereas these were minimum (71.43%) in both *C. catla* and *L. rohita* whereas normal circuli were observed maximum in both *C. catla* and *L. rohita* (28.57%) and it was minimum (23.81%) in *C. mrigala*. In case of radii maximum deformities (85.71%) in *C. mrigala* whereas these were minimum (71.43%) in *C. catla* whereas normal radii were observed maximum in *C. catla* (28.57%) and it was minimum (14.29%) in *C. mrigala*. The anterior margins showed maximum deformities (85.71%) in

*L. rohita* whereas these were minimum (76.19%) in *C. mrigala* whereas normal anterior margins were observed maximum in *C. mrigala* (23.81%) and it was minimum (14.29%) in *L. rohita*. The posterior margins showed maximum deformities (71.43%) in *L. rohita* whereas these were minimum (57.14%) in *C. catla* whereas normal posterior margins were observed maximum in (42.86%) *C. catla* and it was minimum (28.57%) in *C. mrigala*. The *C. mrigala* showed maximum %age of deformities of annuli, circuli, radii & posterior Margin whereas *L. rohita* showed maximum %age of deformities of focus & anterior margin.

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**ACUTE TOXICITY OF CADMIUM AND COBALT TO THE FISH, *CHANNA MARULIUS*,  
*MYSTUS SEENGHALA* AND *WALLAGO ATTU***

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Acute toxicity tests played a significant role in sustainable management and conservation of fish natural aquatic habitats. *Channa marulius*, *Mystus seenghala* and *Wallago attu* are the fast growing high priced carnivorous fish species. However, over the last few decades these **fish species have** suffered substantial decline due to higher contamination rate of natural habitats and overexploitation as a food all over its array that have caused hostile impacts on the growth and well beings of the fish that seems a major cause of their decline in natural habitats. In order to develop plans for their sustainable conservation in the aquatic bodies, it is necessary to define their tolerance limits against persistent metallic ion pollutants like Cd and Co that may impose genetic injury to the natural populations of these fish. Static bioassay tests, under controlled laboratory conditions, were conducted to assess the toxicity of cadmium and cobalt by exposing three length groups of each fish species viz. *Channa marulius*, *Mystus seenghala* and *Wallago attu* to various concentrations of Cd and Co. The 96-hr LC<sub>50</sub> and lethal concentration of each metal was used to determine the quantifiable factors like sensitivity, survival and mortality of the fish. Significant differences existed among three fish species and length groups for their sensitivity that was determined in terms of 96-hr LC<sub>50</sub> and lethal concentrations of Cd and Co. *Wallago attu* appeared significantly more sensitive while that of *Channa marulius* were least sensitive to the toxicity of metals. The 50mm length groups of all the three fish species were significantly more sensitive to metals, followed by that of 100mm and 150mm groups. The 50mm length groups of all the three fish species exhibited significantly lower sensitivity towards cobalt.

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**GROWTH PERFORMANCE OF RED TILAPIA FED WITH INCORPORATED FEEDS  
WITH SEAWEED AND OYSTER**

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Fish feeds supplemented with ingredients of plant origin, effective and less expensive, is demand of aquaculture industry. Aquatic macrophytes and marine organisms are suitable unconventional source of protein to develop ideal fish feed. In the present study nine different feeding trials with different concentrations (2.5%, 5.0% and 7.5%) of hydrolysates of seaweed and oyster were used to observe the specific growth rate (SGR), relative growth rate (RGR) and feed

conversion ratio (FCR). Red tilapia fingerlings with initial weight of  $11.5 \pm 1.00$  gm were fed (6% body weight) twice a day, for 90 days. The maximum weight of 66.67 gm was observed in fishes fed with oyster hydrolysate followed by 65.13 gm fed with seaweed extract. The SGR was calculated separately for each group at different intervals of experimental period. The lowest values of 2.55 and 2.59% of specific growth were recorded in fishes fed with hydrolysate (2.5%) of seaweed and oyster. A maximum specific growth rate (3.53%) was in fishes fed with seaweed hydrolysate (7.5%) as compared to the control (3.42%) and oyster (3.48%) hydrolysate (5.0%). While RGR showed, on day 90, the maximum increment of weight 16.03 gm fed with seaweed hydrolysate (7.5%) as compared 13.48 gm fed with oyster hydrolysate (5.0%). The difference in weight increment among these two groups was 15.90%. However, the increment of weight was relatively higher (17.14 gm) in ethanolic extract of seaweed ( $p < 0.05$ ) as compared to ethanolic extract of oyster (14.02 gm). The maximum average FCR value  $2.27 \pm 0.2$  was recorded among fishes fed with feed supplemented with seaweed hydrolysate (2.5%), whereas, the lowest ( $0.66 \pm 0.3$ ) in seaweed hydrolysate (7.5%). In fishes fed with ethanolic extracts of seaweed and oyster, the minimum average value of FCR was recorded in fishes fed with ethanolic extract of oyster ( $0.67 \pm 0.1$ ) whereas the maximum in the control group ( $1.60 \pm 0.3$ ). These results clearly indicated that the feed supplemented with 2.5% ethanolic extract of oyster showed better efficiency in terms of FCR ( $p < 0.05$ ). The results of this study clearly demonstrated that incorporation of ethanolic extract in commercial feed as compared to other extract helped significantly ( $P < 0.05$ ) in enhancing the growth of the red tilapia.

#### MARKER BASED STUDIES ON POPULATION STRUCTURE OF *Oreochromis mossambicus* IN RIVER CHENAB

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Mozambique tilapia (*Oreochromis mossambicus*) is an important food fish in developing countries. Population structure and genetic diversity of *O. mossambicus* was studied in the Chenab River (Punjab). A total of four SSR (Simple Sequence Repeat) markers were used to study five populations of *O. mossambicus*. The results showed moderate level of genetic diversity. A total of 125 samples, twenty five from each site were analyzed. The number of alleles per locus ranged between 4 and 9 with an average of 6.4. Observed heterozygosity ( $H_o$ ) ranged from 0.586 to a maximum of 0.720. For all the tested locus population combinations, significant deviations ( $P < 0.01$ ) from HWE (Hardy Weinberg Equilibrium) were observed. AMOVA (Analysis of Molecular Variance) indicated that majority of the variance lies within individuals (55.59%) than among individuals within populations (27.98%). In the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram clustering pattern of populations was observed. Results indicated that all the populations are reproductively isolated due to anthropogenic activities and long geographical distance except MH (Marala Headworks) and KH (Khanki Headworks) populations. The MH and KH populations genetically less differentiated due to less geographical distance (58km) and less human interventions. These results can be beneficial in effective fisheries management of tilapia populations.

### EFFECT OF CHRONIC WASTEWATER EXPOSURE ON THE HEALTH OF *LABEO ROHITA*

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The effect of chronic wastewater exposure on the health of fish, *Labeo rohita* was studied. Fish were exposed experimentally to wastewater taken from saggian drain of river Ravi, for 28 days under semi-static laboratory conditions. The analysis of heavy metal levels in wastewater collected from drain of River Ravi revealed that mean value of Mg and Cu in the wastewater samples were  $3.11 \pm 0.04 \text{ mgL}^{-1}$  and  $0.17 \pm 0.05 \text{ mgL}^{-1}$ , respectively. Presence of heavy metals in the wastewater followed the order  $\text{Mg} > \text{Cu}$ . The condition factor (K) of the treated fish used for the visual health assessment of fish was found to be lower ( $0.90 \pm 1.03 \text{ gcm}^{-3}$ ) indicating poor fish health as compared to higher value of  $0.98 \pm 1.72 \text{ gcm}^{-3}$  for control fish. No histological abrasions were found in the liver of control fish. The histological abrasions like hemorrhage, partial degeneration of hepatic mass, hepatic necrosis, alarm cells present and degeneration of vacuole with percentage prevalence of 93%, 80%, 66%, 60% and 53%, respectively were examined in effluent treated group. The mean value of histological alteration index (HAI) for the liver was significantly high and reported as 4.3. The higher value of histological alteration index depicted need of wastewater treatment prior to be used for fish culture so that negative impact of wastewater upon fish health can be lessened.

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### A COMPARATIVE STUDY ON GROWTH PERFORMANCE OF CHINESE CARPS BY REPLACING SOYBEAN MEAL WITH DUCKWEED AS DIETARY PROTEIN SOURCE

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Development of ecofriendly and efficient fish feed for best growth performance is an important step in the field of fisheries and aquaculture. A feeding trial was conducted to compare the effect of Duckweed (*Lemna minor*) and soybean meal as a source of protein on the growth performance, growth hormone level and proximate composition of *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* in monoculture system and in combination. Two 35 % protein experimental diets,  $F_{\text{SBM}}$  containing 21 % soybean meal and  $F_{\text{DW}}$  having 21 % duckweed as a source of protein were prepared and fed for 12 weeks. At the end experiment, advanced fry of *Ctenopharyngodon idella* showed a significantly higher growth rate as compared to *Hypophthalmichthys molitrix* when fed  $F_{\text{DW}}$  while *Hypophthalmichthys molitrix* showed a higher growth rate in response to  $F_{\text{SBM}}$  diet in both culture systems. A similar trend was observed in Percentage weight gain (% WG), specific growth rate (SGR %) and feed conversion efficiency (FCE %). The growth hormone level of advanced fry of *Ctenopharyngodon idella* fed  $F_{\text{DW}}$  were significantly ( $P < 0.05$ ) higher in both culture systems while same trend was shown by *Hypophthalmichthys molitrix* when fed  $F_{\text{SBM}}$  diet. Proximate analysis showed approximately similar composition of both diets. The results of this study indicate the use of duckweed as a protein source in fish feed.

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**DEVELOPMENT OF VALUE ADDED PRODUCTS, QUALITY DETERMINATION,  
NUTRITIONAL AND ORGANOLEPTIC ASSESMENT FROM *CHANNA MARULIUS*  
FLESH**

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*Channa marulius* inhabits freshwater swamps, slow moving water and fissures or vicinity of riverbanks of all the tributaries of rivers in Pakistan. The fish have a therapeutic value and helps in wound healing during accidental major and minor cuts. Its flesh contains omega-3 polyunsaturated fatty acids, which serve as precursors to prostaglandins and wound healing proficiency. Due to high requisition and consumer preferences, market prices of this fish are always much higher than herbivorous varieties. Based on various traditional value added fish products in the market, sausages are specially suitable on the basis of organoleptic and technological characteristics replacement of its flesh by the sausage. In this study, sausage from Murrels fish (*Channa marulius*) was prepared. The purpose of study was to determine sausage made from Saul (*Channa marulius*) flesh was consumable or not. In order to find out quality characteristics of fish paste, fish flesh and fish fillets, physical analysis, chemical analysis and organoleptic test were determined. No significant differences for pH were observed among the fish paste, fish flesh and fish fillets products ( $P>0.05$ ). Due to similar studies; the quantity of separated fat, water holding capacity and loss of weight caused by cooking method were found low in paste of sausage while process efficiency was found high. In addition, chemical analysis of protein, moisture, and fat amounts were not higher than the required values. In conclusion, the quality assessment results indicated that *Channa marulius* flesh could be used for sausage processing on the commercial level.

**CHEMICAL QUALITY ASSESMENT OF MINCE BASED VALUE ADDED PRODUCTS  
FROM MORAKHI (*CIRRHINUS MRIGALA*) DURING FROZEN STORAGE**

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Value addition is the most capable and developed sector in food processing industry. With the advanced technology the modern methods of processing and preservation have encouraged the consumption of many other such species of fish that are not so popular throughout the world. Mincing provides a chance to apply greater control over taste, appearance and keeping quality by the integration of additives. The developed fish minced products such as fish nuggets, kebab, cutlets, etc, are safe, secure and hygienic for human consumption. Such value added products are

also liked by a large number of consumers globally. The increasing demand for ready to eat fish products in developing countries especially in Pakistan, there is a need to diversify our freshwater food and mined based value products that belong to such technology for diversification. Morakhi (*Cirrhinus mrigala*) weighing  $1.5 \pm 0.02$  kg were deboned and prepared the mice with machines. There were four freezing intervals i.e. 8<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup> & 32<sup>nd</sup> days to evaluate shelf life of the products. The value added products (fish cutlets, fish nuggets, fish kebab, fish balls and fish patties) were prepared by using different feed ingredients. The recipes for the products were selected by comparing the acceptability of different formulations of ingredients by sensory, proximate, microbial and statistically. The results showed that the sensory evaluation were non-significant. The odour of fish kebab, fish nuggets, fish balls, fish cutlets and fish patties was fresh during whole frozen period. These products were liked extremely during whole storage period. Proximate analysis showed significant results ( $P > 0.05$ ). The chemical analysis of the value added products showed that crude ash content of fish kebab during frozen storage were  $3.16 \pm 0.01$ ,  $3.04 \pm 0.13$ ,  $2.80 \pm 0.02$  and  $2.03 \pm 0.04$  at 8<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup> & 32<sup>nd</sup> days respectively. The moisture content of fish patties was also increased. In fish patties, fish nuggets, fish balls, fish cutlets moisture was increased while in fish kebab moisture was decreased. In conclusion all the products were highly acceptable at 8<sup>th</sup> day interval as it retained best protein, fats, moisture and ashes content. Besides this at 8<sup>th</sup> day of interval the content of TVBN, TBA, FFA and PV are also at their minimum thus enhancing its chemical quality and making it more hygienic for human consumption.

#### **STUDIES ON BIOLOGICAL ASPECTS OF FRESHWATER CATFISH *MYSTUS BLEEKERI* (DAY, 1877) FROM LOWER INDUS RIVER, THATTA, SINDH, PAKISTAN**

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*Mystus bleekeri* (Day, 1877) commonly known as Tengra (or gulsha). It is commercially important catfish of Pakistan. It is locally found in rivers, large canals and other freshwater bodies in Pakistan, India, Bangladesh, Nepal and Indonesia (Talwar and Jhingran, 1991). The fish specimen from Thatta fish market on monthly basis from February to August however, during the November to August *M. bleekeri* was captured in low quantity by fishermen due to increase in river water due to flood in Indus River. The parameters *a* and *b* were estimated by linear regression on the log transformed equation:  $\log W = a + b \log L$ . The fish sample comprised 160 male and 207 female being 367 in total. Size of female *M. bleekeri* ranged between 7.1-15.8 cm and the size of male *M. bleekeri* ranged between 8.1-14 cm. Values of *b* for male, female and combine sexes were calculated as (2.08, 2.22 and 2.22), indicating the the negative allometric growth for male, female and combine sexes. It was also observed that the relationship between length and weight of *M. bleekeri* was linear with strong positive relationship. Male female ratio was 1:1.26 where females were larger than males. Sexual dimorphism was evident where males were recognized through availability of prominent papilla whereas females did not possess such papilla. Gonado-somatic index (GSI) started to increase in March, reaching at peak in April, remained higher till August, started decreasing in September. Indicating the single spawning season in summer.

### ABUNDANCE AND SPATIAL DISTRIBUTION OF ZOOPLANKTONS IN THE MANGROVE FORESTS DURING SOUTHWEST MONSOON SEASON

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Zooplankton include all floating animals and are found in abundance in waters where abundant food is present. They migrate vertically and horizontally according to the intensity of the Sun light. Mangrove forest ecosystems are the second most productive ecosystem in the world. In Pakistan mangroves are found along the coast of Sindh and Baluchistan. In mangrove ecosystems, zooplankton forms a fundamental trophic link in aquatic food webs. Most of zooplanktons like Copepods serve as prey for many juvenile mangrove fish due to their dominance, relatively small-sized and availability. The samples for the estimation of zooplankton abundance in mangrove forests were collected through zooplankton net. Zooplankton abundance was estimated by counting in zooplankton counting chamber under binocular. The phytoplankton biomass will be estimated through chlorophyll analysis. Over all the copepods was the dominant group in abundance followed by crustacean larvae. Cluster and MDS analysis of zooplankton abundance based on phytoplankton biomass indicates similarity between different SW-Monsoon periods.

### STUDIES ON BIOLOGY OF FRESHWATER SILURID CATFISH *OMPOK PABDA* FROM INDUS RIVER, JAMSHORO, SINDH, PAKISTAN

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Catfishes of the genus **Ompok**, are medium-sized members of the Siluridae family usually found in lakes and large rivers throughout South and South-East Asia (Ref). The catfish **Ompok pabda** (Hamilton, 1822) locally known as Pabdah (butter fish) is an indigenous small freshwater fish belonging to the family Siluridae of the order Siluriformes. It is a highly priced delicious catfish and well preferred because of relatively few bones. Monthly fish samples were collected from February 2015 to July 2015 through the catch of fisherman from Indus River, Jamshoro. The fish sample comprised of a total 261 specimens (88 male fish and 173 female fish) having total length (TL) 9.2- 21.5 cm (males) and 10.7- 30.0 cm (females), weighing 8.4- 48.2 g (males) while females were 6.4- 147 g. The LWRs estimated by linear regression were  $\text{Log } W = 0.135 + 1.857 \text{ Log } L$  (for male),  $\text{Log } W = 0.533 + 0.292 \text{ Log } L$  (for female) and  $\text{Log } W = -1.615 + 2.520 \text{ Log } L$  (for combine population) indicating negative allometric growth. The food analysis studies revealed that the diet of *Ompok pabda* contained fish meat, semi digested fish meat, fish spines and crustaceans. The fish were categorized in two length groups, small fish and large fish group to check the ontogeny. The small fish (10-16 cm) preferred 26% fish meat, 61% semi digested fish meat, 3% fish spines and 1 % crustaceans. The second length group, large fish (17-30 cm) they mostly preferred 24% fish meat, 55% semi digested fish meat, 2% fish spines and 7 % crustaceans were present inside their stomach also. Small fish group (10-16 cm) was found more voracious as compared to large fish group (16-30 cm). The breeding season was from April to May, where April

was found peak season. Gono-somatic Index (GSI) showed continuous rise from February to March while decreased up till April to May, again in June it rises but decreases again in July. Egg size ranged from 0.22 mm (Feb) to 0.90 mm (April).

**A TRIAL PRACTICE OF ALGAE CONTAINED FORMULATED FEED APPLIED TO COMMON CARP *CYPRINUS CARPIO* AT MANDO DERO FISH HATCHERY SUKKUR SINDH PAKISTAN**

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The 2000 fry of *Cyprinus carpio* length sizes 1-1.5 cm and weight 0.42- 0.67g were stocked in five nurseries (400 in each nursery). The algal pallet feed 5% per body weight was applied. Growth rate of fish length and weight was noted on the monthly basis. The trial was started from April as first month the growth rate of fish was found 4.40g and length 4.2 cm, in May 12.50g and 8.6 cm, June 95.30g and 12.5 cm, July 210.40g and 16.1 cm, August 290.90g and 20.5 cm, September 630g and 24.7 cm, October 940g and 28.1 cm and November 1250g and 34.5cm respectively. Algal biomass was collected from nurseries, it was sun dried, cursed and converted in floor then Mustard oilcake, Wheat flour , Rice flour and Water was added the mixture was brought into local machine for preparation of fish feed complete form. Algal pellet feed is comparatively cheap, non poisonous and rich protein contained. Fish growth rate and FCR were observed ideal.

**WEIGHT-LENGTH RELATIONSHIP, CONDITION FACTOR AND FISH POPULATION ESTIMATION OF BALLOKI HEADWORKS ON RIVER RAVI AND SULAINMANKI HEADWORKS ON RIVER SUTLIJ, PAKISTAN**

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The weight-length relationship is an important aspect for the management of fish population. Fish size is too much affected by conditions of environment, as food and temperature etc. Fish's weight and length also change with the passage of time and also within different locations, it depends on the food quantity reproduction activity and competitors. The population of the fish has been declined to a large scale due to urbanization and agricultural processes, fish exploitation and eutrophication. Many fish specie's size is small. The distribution of different fish species also depends on conditions of environment such as velocity, substratum's nature, and quantity of food and quality of food. This study was assumed to reveal information on the length weight relationships & condition factor (Kn) of freshwater fishes. *Cyprinus carpio*, *Labeo rohita*, *Cirrhinus mrigala*, *Catla catla*, *Hypophthalmichthys molitrix*, *Ctenophryngodone idella*, *Channa marulius*, *Rita rita*, *Aorichthy aor*, *Walago attu* and *O. mossambicus* and many other fresh water

fish species were found in abundance on both study sites in same proportion. However, *Cyprinus carpio* and *Labeo rohita* were highest in number whereas *Channa murulus* was least on the base of numbers. This study was conducted by investigating many specimens including both male and female collected from various sites of Head Baloki, such as, Khan Ki pull, Khokhar nagar, Baloki Briedge and Baloki Camp and also from various sites of Head Sulimanki such as, Dharaga, Kalay walay Bachni, Manga Thakre ka and Dara Dona from September, 2014 to February, 2015. Results showed almost isometric growth which indicates that the small specimens have same form and condition as of large specimens. It is also evident that pronounced seasonal fluctuations in ecological conditions result changes in condition factor due to feeding and seasonal cycles. The relative condition factor showed variation in all size groups. The males and females revealed significant differences in the value of 'a' and 'b' however, relative condition factor (Kn) did not vary significantly. Males recorded higher exponential value. High 'b' values in case of females were also observed.

#### **EFFECT OF TOURISM LOAD ON FISH POPULATION IN RIVER KUNHAR MANSEHRA KPK, PAKISTAN**

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The aim of the present investigation was to determine the effect of tourism load on population of fish fauna in river Kunhar. For this purpose seven different sites were selected at river Kunhar which were important for tourism. *i.e.*, Lake Saif Ul Maluk; Naran; Kaghan; Jared; Kiwai; Balakot and Gari Habibullah. The study was conducted on three tourism periods *i.e.*, Pre tourism (April and May), Peak tourism (Jun, July and August) and Post tourism (September and October). Fishes were caught with the help of cast net by local fisherman and then immediately preserved in 10% formalin solution. A total of 2034 fishes were collected from the seven sites of the river during these three tourism periods out of which 937 fishes were collected in Pre tourism period, 419 fishes were collected in Peak tourism and 678 fishes were collected in Post tourism. From the current study, it is concluded that tourism load badly affected the population of fish fauna via anthropogenic pollution. Lowest population was recorded in peak tourism season. If the tourism activities on these sites are not regulated properly, so in future very soon the fish population will be affected badly.

#### **NEOPLASIA DUE TO TOXIC EFFECT OF TREMATODES IN SOME EDIBLE FISHES OF KARACHI COAST**

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Fishes have socio-economics position in all over the world particularly marine fish give a bulk of food for mankind even as fishes of the lakes and rivers are also important source for the food supply. Fish provide a high protein, low-fat, which is low in saturated fats. Now a day, due to

different disease such as diabetes, cardiovascular problems, hypertension, osteoporosis, osteomalacia, ocular diseases etc., and doctors recommend white meat as compared to red meat. Fish is also recommended for people suffering from malnutrition. Fish production is an important source of income and employment and plays a major role in Pakistan's economy. Its organ such as liver is an important source of oil and has medicinal value and is used for the treatment of different diseases. Fish also provide fish fertilizer, fish-manure and several other products of commerce. Some fish take pollutants from the environment and water in which they live in and the food they eat. Some of these contaminants provoked in the form of tumorous growth. The word tumour has often been used in a general way to signify any hump or swelling. But commonly the word tumour define as a new growth of cells which deviated from normal surroundings cells in shape, size, structure and organization. The present study deals with neoplastic growth and as well as trematode infection. The intensity of infection was noted for the whole period of investigation from January 2009 to December 2012. During the study 2,373 species of fish were examined, out of which 1,178 were found to be infected by helminthes. Mostly the trematodes and neoplastic growth were observed in fish *Johnius dussumieri* (Valenciennes, 1833) called as "Mushka" of Karachi coast. In case of trematodes the maximum prevalence was recorded during the hot (summer) season of the period while the minimum prevalence was observed in cold season. There were such a great variations observed during the period of study especially in case of neoplastic growth which varies in all the months. Statistical analysis was carried out by Descriptive Statistics, Descriptive measures and Analysis of variance and Duncnan's Multiple Range Test which revealed that there were significant difference in the monthly results of mean intensity of trematodes and as well as neoplastic growth. Grosspathological study shows that tumour was separated from the surrounding organs and showed no sign of proliferation. No haemorrhages were observed on the tumour or around the organs. Histopathologically the tumour shows hyperplasia, malpighian cells, atrophied cells, high prismatic basal cells, and fibrosis with eosinophilia and inflammatory cells infiltration. It is concluded that presence of trematode infection not only is responsible for various types of histopathological changes on the fish individually but it may also responsible to provoke biochemical changes to promote tumorous growth in most of the fishes under study. But some of the fishes under study shows only tumorous growth without any trematode infections which may be due to some pollutants which contaminate the water or the surrounding environment which are responsible for such type of growth in fishes.

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**A COMPARATIVE STUDY OF GROWTH WITH DIFFERENT PROTEIN LEVELS IN ROHU (*LABEO ROHITA* HAMILTON, 1822)**

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The effect of various levels of crude protein (15%, 20% and 25% CP) supplemented diets on the growth rate, feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) was studied for three months on Rohu (*Labeo rohita*). It was hypothesized that the growth response of the fingerlings of Indian major carp, Rohu, would be better with higher levels of protein in diets than the commonly used diet, Rice bran (control diet, having 8.1% crude protein) in semi-intensive pond culture. For the control and each experimental diet a triplicate group of ponds was selected at carp hatchery and training center, Peshawar. The fingerlings of Rohu were

fed on the supplemented diets @ 4% of their body weight. The stocking density was kept 2000 fingerlings/ acre. The sampling was done on monthly basis. The fertilization of the pond and water quality were monitored on a regular basis. The results of the weight gain and proximate body composition with supplementary diets were highly significant ( $P < 0.05$ ) in comparison with that of the control diet. The FCR value was the lowest against the 25% CP diet while it was highest with the control diet. Rohu gained the highest percentage of crude proteins with 25% CP diet as compared to 15% and 20% CP diets. The results of the experiment showed that Rohu showed better growth with 25% CP followed by 20% and 15% CP diets. Hence feed containing 25% CP was found to be satisfactory for fish growth instead of 15% and 20% CP. Technical support of NARC (Fisheries section) and financial support of PARC, Islamabad is highly acknowledged and appreciated for this project.

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#### HEMATOLOGICAL PARAMETERS IN FARMED FISHES FROM LOWER SINDH, PAKISTAN

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For screening hematological parameters, blood samples from the experimental fish were collected monthly during March to August 2013 from Thatta and Badin. Total 150 blood samples from different fish species like *Cyprinus carpio*, *Rita rita*, *Tilapia nilotica* and *Oreochromis mossambicus* were collected and preserved in sampling bottles for subsequent studies. The hematological parameters of four different farmed fishes such as Gulfam, *Cyprinus carpio*, tilapia, *Oreochromis mossambicus*, *Tilapia nilotica* and Khagga, *Rita rita* from Thatta and Badin districts were studied from March to July 2013. The variation in different hematological parameters indicated that hemoglobin concentration found to be significant highest in *Tilapia mossambicus* (9 g/100 ml) and lowest in *Rita rita* (5.8 g/100 ml). The rate of Erythrocyte Sedimentation Rate (ESR) was found to be highest in *Cyprinus carpio* (4.6) and lowest in *Rita rita* (2.7). While Erythrocyte and leukocyte counts were in *Tilapia nilotica* ( $4.02 \times 10^6 \text{mm}^3$ ), *Oreochromis mossambicus* ( $38.31 \times 10^6 \text{mm}^3$ ), and *Cyprinus carpio* ( $2.06 \times 10^6 \text{mm}^3$ ) *Tilapia nilotica* ( $27.68 \times 10^6 \text{mm}^3$ ) highest and lowest respectively.

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### 3. MARINE BIOLOGY

#### SOME OBSERVATIONS ON THE DISTRIBUTION, ABUNDANCE AND POPULATION PARAMETERS OF SHORTFIN MAKO (*ISURUS OXYRINCHUS*) IN THE OFFSHORE WATERS OF PAKISTAN

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Shortfin mako (*Isurus oxyrinchus*) belonging to Family Lamnidae caught mainly by pelagic gillnets in the coastal and offshore waters of Pakistan. In the offshore gillnet and longline fisheries, shortfin mako was observed to be the most dominating species of shark. This species is ranked as vulnerable species according to IUCN Red list and there is a general concern over their commercial harvesting in Pakistan and other countries. Meat of this species like other sharks is consumed locally whereas its fins are exported mainly to Hong Kong. Considering their vulnerability, data about various aspects of their fisheries was collected from October 2012 to October 2015 which revealed that this species is predominantly found during November to April with peak in March whereas a minor increase in catch during December and January. Its main fishing grounds are located between 50 to 200 m depth all along the coastline. Its size ranges between 35 and 215 cm with 50-100 cm as dominating size cohort. Based on the size frequency data various population parameters were calculated which indicates that its stocks are under serious stress because of fishing mortality. Maximum size ( $L_{\infty}$ ) and size at birth ( $L_0$ ) were calculated to be 305 cm and 84 cm respectively. Growth coefficient ( $k$ ) was calculated to be 0.073 and sex ratio was 1.14:1 (M:F). The species was found to feed on variety of bony fishes, other sharks, cephalopods whereas large individuals may feed on larger prey such as billfish and small cetaceans. Although limited information about its breeding is available, however, the species was observed to be reproductively active during winter months (November to February).

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#### THE QUANTITATIVE ANALYSIS OF MAJOR GROUPS OF MACRO-FAUNA (GASTROPODS, BIVALVES, POLYCHEATES, CRABS) IN THE MANGROVE CREEKS OF HAJAMBRO, INDUS DELTA, PAKISTAN

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The present study showed that the distribution of fauna in mangroves at Indus delta does not appear to be regulated as function of vegetated and non-vegetated areas similar to what has been observed in some earlier studies, but showing mix results. The values appear to be quite low that were recorded from sites with dense mangroves and high pneumatophores density. However, abundance of high macrobenthos was observed at site which lacked pneumatophores but had *Oryza* sp. vegetation with high density of burrows. High abundance of fauna (gastropods, polychaetes, crabs) at site with *Oryza* sp. vegetation on mudflats is in good agreement some studies, although contrary to some other findings. Since this study has been conducted in summer (May/June) only



due to inadequate available facilities, and therefore it offers only limited value in ascertaining effect of, for example, other mangrove species (*Aegiceros corniculatum*; other than dominating (99%) species, *A. marina*), other vegetation (grasses; *Oryza* sp.) inhabiting the area.

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#### **A RISK AND BENEFIT ASSESSMENT APPROACH ASSOCIATED WITH SEAFOOD CONSUMPTION**

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The extent of contamination has increased markedly in the last 50 years due to technological developments and increased consumer use of materials containing heavy metal. Increased levels of heavy metals such as cadmium (Cd) and lead (Pb) in seafood constitute a food safety risk to consumers. Shellfish are an important source of toxic metals, but also of essential elements in the diet. Seafood provides long-chain omega-3 fatty acids, vitamins and minerals, which are essential to maintain good health. Moreover, seafood is a source of contaminants such as heavy metals and persistent organic pollutants that may affect health. In this study the level of Zn, Cu, Cd, Pb and Cr have been analyzed in fresh shellfish products. In order to limit exposure, while, maximizing the benefits of seafood consumption, quantitative and qualitative risk-benefit analyses have been conducted for seafood consumption. The potential human health risk and nutritional value of the product analyzed were also assessed.

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#### **MARINE POLLUTION IN COASTAL AREAS OF PAKISTAN**

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Pollution of coastal ecosystem by chemical substances such as hydrocarbons (oil spills, wastes), pesticides (crop treatment), heavy metals and various organic pollutants is a major environmental concern. Pollution is discharged directly into the sea, or enters the coastal waters through rivers and by atmospheric deposition. Widespread use of polychlorinated biphenyls in various industries has led to environmental contaminations. Polychlorinated biphenyls are generated in the emissions of industrial plants, thermal power stations, vehicular traffic, road infrastructures, and agricultural practice. PCB accumulates in fatty tissues and their concentrations increase ten fold at every link of the food chain through the process of bioaccumulation. High sensitivity of marine organisms to these pollutants may have direct effect on the fish diversity. Polychlorinated biphenyls (PCB) were analysed in soil samples from coastal areas of Pakistan. Polychlorinated biphenyls screening kit were used for analysis. The result shows that PCB contamination of soil is 62% in five stations. National legislation, policies and plans encouraging toxic waste minimization and waste recycling and use are important for healthy environment. It is necessary for the Government and industry to undertake a number of activities as part of the comprehensive toxic wastes management effort, leading to the establishment of treatment and disposal facilities. It is necessary to prevent, combat and control marine pollution. Industries should review their waste disposal practices with the aim of minimizing and eventually eliminating

harmful waste discharge. The national and international authorities should regulate this activity and to enact and strictly enforce environmental laws and regulations based on the precautionary and polluter pays principles.

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**DIVERSE PATTERNS OF MORPHOLOGICAL AND GENETIC VARIABILITY IN THE POPULATIONS OF THE THREE SYMPATRIC SPECIES OF FIDDLER CRAB OF GENUS *UCA* (CRUSTACEA: DECAPODA: BRACHYURA) ALONG THE COAST OF PAKISTAN**

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Preceding study has recognised wide-ranging inter-population variability in the morphology of the three species of *Uca iranica*, *Uca sindensis* and *Uca annulipes*. To determine the source of inter-population variation (genetic or environmental) of each species of *Uca*, morphological and genetic data were investigated from crabs collected from 4 sites (Sandspit, Korangi, Sonari and Sonmiani) along the coast of the Pakistan. Nine morphometric characters (carapace length, carapace width, wet weight, enlarged chela length, enlarged chela width, abdominal length, abdominal width, and pleopod length) were measured from each species of fiddler crabs and the degree of morphological similarity among sites was designed using multivariate techniques. Isozyme electrophoresis was used to examine diverse patterns of genetic similarity. Wide spread intra-species morphological variability was detected: seven out of the nine morphometric traits were useful when discriminating between crabs from each site. Discriminant function analysis revealed that over 35% of individuals could be classified to their site of origin on the basis of their morphology. In contrast, the isozyme analysis revealed low levels of genetic variability, both within the inter-population and among the crab population at each site. Pairwise comparisons revealed a reasonable correlation between the degree of morphological and genetic similarity of crabs at each site, which suggests that the observed phenotypic variability has a genetic component. However, only around 20% of the phenotypic variability detected was associated with the patterns of genetic similarity. This means that patterns of morphological variability in this species are largely determined by the local environmental conditions: confined factors could have a within generation selective impact on mean trait values or genus *Uca* may exhibit phenotypic flexibility.

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**DAY-NIGHT DISTRIBUTION, COMPOSITION AND ABUNDANCE OF ZOOPLANKTON GROUPS IN WATERS ALONG KARACHI COAST, PAKISTAN**

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Day and night distribution, composition and abundance of the main zooplankton groups were analyzed during the year of 2008-9. The copepods, comprising 64.95% in day time and 54.97% in

night time at St.1 of the whole zooplankton groups and 73.91% in day time and 64.41% in night time at St.2 of the whole zooplankton population were the most abundant followed by the Appendicularia 20.31% in night and 20.44% in day time at St.1, 18.18% in night time and 14.13% in day time at St.2, Cladocera 18.50% in night time and 13.30% in day time at St.1, 15.61% in night time and 10.80% in day time at St.2. Generally, the day and night composition at both stations (St.1 and St.2) showed little different pattern, most of the zooplankton groups were present at a slightly higher abundance at day time. The Copepods abundance was highest during the month of April in day time and during the months of October and November in night time, other zooplankton groups also shows their high abundance during the same months. The zooplankton diversity ( $H'$ ) at St.1 was 1.567-1.781 in night time and 1.069-1.532 in day time whereas at St.2 it was 1.227-1.626 in night and 0.888-1.51 in day time. There is slight difference between the day and night abundance and composition of different zooplankton groups. This study reveals that coastal waters of Karachi are rich in zooplankton population and their abundance enhances the fishing activities.

#### GENETIC RELATIONSHIP AMONG SENTINEL CRABS (DECAPODA, BRACHYURA, MACROPHTHALMIDAE) FROM MANGROVE AREAS OF PAKISTAN

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Sentinel crabs of family Macrophthalimidae are one of the most dominant macrobenthos in intertidal mudflats of tropical and subtropical shorelines. The studies were carried out to investigate the level of the genetic differentiation among four species of macrophthalid crab, collected from different coastal areas of Pakistan. To estimate genetic variability polyacrylamide gel electrophoresis (PAGE) were used to screened three different enzyme system including Catalase (CAT), Carbonate dehydratase (CD), Peroxidase (PER) and a general protein (G.P). Out of 18 loci examined 8 (G.P IV, G.P IV, C.K II, CD II.C.D III, CAT II, CAT IV, PER 1) were polymorphic and remaining monomorphic. The percentage of polymorphic loci was ranged from 0.05 to 0.22 with an observed heterozygosity ranged from 0.045 to 0.139.

#### SOME PHYLO-GEOGRAPHICAL STUDIES OF *CHARYBDIS FERIATA* (LINNAEUS, 1758) IN INDO-WEST PACIFIC WATERS, AS INFERRED FROM MITOCHONDRIAL DNA EVIDENCE

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Swimming crabs (family Portunidae Rafinesque-Schmaltz, 1815) are crustaceans those inhabit sub tidal, estuarine and offshore waters, and are widely distributed across the Indo-West Pacific region. *Charybdis* De Haan, 1833, is the second largest genera within the sub-family *Thalamitinae* Paulson, 1875, with 63 species. Mitochondrial DNA (mtDNA) sequence analysis was

used for phylogeography of *C. feriata*. During the present study the amplification of partial sequences of mitochondrial gene from representatives of *C. feriata* was performed, for examination along with the representative sequences of (India, China, Vietnam and Taiwan) was obtained from Gene Bank. Results were confirmed by searching for sequence similarity using BLAST n (Basic Local Alignment Tool), the result was showed 99% sequence similarity with Indo-West Pacific populations, gene bank accession no: KF 220503.1-KF 220506.1, KF 386147.1 (India), KF 386147.1, AY 497291.1, DQ062727.1 (China); AM 410535.1 (Vietnam) and KJ 132522.1 (Taiwan). The obtained sequences of local population also submitted to Gene Bank. An evolving relationship of *C. feriata* was inferred using the neighbour-joining method and estimation of evolutionary divergence analysis between populations of *C. feriata* was also conducted using Molecular Evolutionary Genetics Analysis (MEGA 6).

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#### GUT CONTENT ANALYSIS OF *CHARYBDIS FERIATUS* IN NATURAL ENVIRONMENT

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*Charybdis feriatus* possess long sharp-toothed chela, which helps them for rapid snapping movements and tear the prey easily. In this study, gut contents and their composition with reference to different size group of *Charybdis feriatus* in various months were analyzed by using the percent occurrence and percent frequency method. Crabs were purchased from commercial fish harbor were immediately preserved in formalin. Stomach contents of 446 crabs were examined of which 226 male and 220 were female. Stomach content mainly consists of crab, fish and molluscs. Despite the diversity of diet and feeding habits *C. feriatus* shows opportunistic omnivores behaviour with a preference for animal food and with predatory tendencies.

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#### INTERTIDAL MACROBENTHIC COMMUNITY STRUCTURE IN MANGROVE SWAMPS AT SANDSPIT BACKWATERS, KARACHI, PAKISTAN (NORTHERN ARABIAN SEA)

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Sandspit backwaters lies about 18 km south-west of Karachi city and contain shallow tidal lagoons, inter-tidal mudflats and 400 hectares mangrove swamps. The western side is comprised of open sandy beach which stretches about 20 km along the Arabian Sea coast. The backwater area at Sandspit, Karachi is densely populated by *Avicennia marina*. Karachi is being faced by pollution from the nearby industries. The present study deals with the distribution of macrofauna community structure in the stressed environment giving an insight of the prevailing health of the mangrove ecosystem in Pakistan. The mangrove swamps is supported by a rich and diverse assemblages of crustaceans, molluscs, polychaetes and echinoderms. *Cerithidea cingulata* was found to be the most abundant organism in the mangrove system. The data was analyzed for a period of two years. Seasonal variation in the benthic composition were also observed. Statistical analyses were

employed to define the health of the mangrove ecosystem at the three monitoring sites at Sandspit backwaters, Karachi.

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**PROTEIN ESTIMATION AND MOLECULAR WEIGHT DETERMINATION OF THE  
*METAPENAEUS MONOCEROS* (FABRICUS 1798) AND *PENAEUS* (*FENNEROPENAEUS*)  
*MERGUENSIS* (DE MAN, 1888) (ORDER: DECAPOD; FAMILY: PENAEIDAE)  
COLLECTED FROM KARACHI FISH HARBOR**

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Proteins are the most versatile macromolecules in living systems and serve vital functions in all biological processes. They function as catalysts, transport and store other molecules such as oxygen, provide mechanical support and immune protection, generate movement, transmit nerve impulses, and control growth and differentiation. The Decapoda are among the better investigated animal groups, which is mainly due to their ubiquity in aquatic ecosystems, their historical role as biological models and their importance as human food. Among decapods penaeid shrimps are commercially important and are native to Indopacific region. To estimate their protein's molecular weight, SDS-PAGE is an unpretentious and subtle method used to fractionate proteins and provide an estimate of their molecular weights. Two species of subfamily penaeini; *Metapenaeus Monoceros* and *Penaeus merguensis* were collected from Karachi fish harbor. Lowery *et al.* method was used to estimate general protein pattern in both species. After morphometry, samples were screened for general protein using SDS-PAGE electrophoresis with known weight of biochemical markers. Relative mobility and frequencies of obtained proteins band were observed to measure molecular weight of general proteins. This preliminary study showed that protein is the most prominent biochemical component of crustacean and varied specie to specie. These variations can be helpful in electrophoretic identification of closely related species.

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#### 4. PALAEOLOGY

##### BOVIDAE FROM THE LATE MIOCENE DHOK PATHAN FORMATION OF SIWALIKS, NORTHERN PAKISTAN MUHAMMAD

AKBAR KHAN AND MUHAMMAD AKHTAR

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The Dhok Pathan Formation of the Middle Siwalik Subgroup hosted a highly impoverished bovid fauna during the Late Miocene to Early Pliocene times. This was a result of its mosaic palaeoenvironments and biogeographic connections, which support the immigration of most Miocene bovids. The main bovid tribe is a extremely diversified Boselaphini, commonly found in many sites across the Late Miocene deposits. The tribe represents small, medium and large size species during the Late Miocene-Early Pliocene. Here we describe assemblages excavated in the deposits of the Chakwal district. Seven bovid species are identified: *Pachyportax latidens*, *P. cf. nagrii*, *Selenoportax cf. vexillarius*, *Tragoportax punjabicus*, *T. cf. salmontanus*, *Elachistoceras khauristanensis* and *Gazella lydekkeri*. The findings enlarge our knowledge on the taxonomic features of the Late Miocene bovids.

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##### MAMMALIAN REMAIN FROM THE NEWLY REPORTED LOCALITY FROM THE PUBBI HILLS OF GUJRAT, PUNJAB, PAKISTAN

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The newly discovered locality is nearby the Panjan Sher Shahana village in district Gujrat and the locality is dominated by the outcrops of the Pinjor Formation. The Formation is dominated by loose coarse sandstone. The Pabbi Hills of northern Pakistan comprise an Upper Siwalik fluvial sequence, ranging from 3.2–0.5 Ma. The Pinjor Formation encompasses ca. 2.5–0.6 Ma of the Indian subcontinent, and was extensively sampled for vertebrate fossil remains. This locality has yielded a diverse mammalian fauna of Plio-Pleistocene. The recorded faunal elements from the locality are discussed here.

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**NEW REMAINS OF *DORCATHERIUM MAJUS* (TRAGULIDAE, MAMMALIA) FROM  
THE NAGRI FORMATION OF THE SIWALIKS, NORTHERN PAKISTAN**

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Tragulids, especially *Dorcatherium*, are the best represented ruminants in the Middle Siwalik Subgroup of the Nagri Formation, northern Pakistan. New remains predominantly consist of isolated teeth and mandible fragment are reported from Nagri type locality, district Chakwal, Punjab, Pakistan. The outcrops are dominated by coarse to medium grained blue massive sandstones and lesser amount of clays, mudstone and siltstones. The material is assigned to *Dorcatherium majus* on the basis of its size. The genus *Dorcatherium* dominated in the Late Miocene of the Siwaliks and became extinct at the end of the Early Pliocene. The tragulids indicate the climatic conditions and landscapes experienced drastic change towards dryer and more open environments.

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## 5. WILDLIFE, DIVERSITY AND CONSERVATION

### HERPETOFAUNAL DIVERSITY OF TOLIPIR NATIONAL PARK (TNP) AZAD JAMMU AND KASHMIR (AJK) PAKISTAN

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The herpeto faunal diversity of the Tolipir National Park (TNP) provide base line data about the species composition and community structure. The herpeto fauna was surveyed in summer, 2013. I recorded 4 amphibian species and 12 reptile species by visual encounter and pitfall trapping. Various diversity indices, *i.e.* Simpson Index (0.13), Shannon Winner Index (2.80), Berger-Parker Dominance Index (0.12), Margalef's Index (2.9), Menhinick Index (0.85), Buzas and Gibson's Index (0.86) were calculated to quantify the herpeto faunal diversity. The study provides base line data for future studies and comparisons of the herpeto fauna of other national parks to develop a preliminary scheme for the conservation of the herpeto faunal diversity.

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### DIVERSITY AND NESTING HABITAT OF OWL SPECIES INHABITING MARGALLA HILLS NATIONAL PARK (MHNP), ISLAMABAD

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Owls, the apex predators, are grouped in two families, namely Strigidae and Tytonidae; family Strigidae is represented by 19 species while Tytonidae by a single species in Pakistan. The current study investigated diversity and nesting habitats of owl species inhabiting the Margalla Hills National Park (MHNP), Islamabad. Surveys were conducted fortnightly in different parts and at different elevations to record owl species and their nests. A total of 14 potential literature defined sampling sites were visited from February 2014 to May 2015. Three owl species (*Athene brama*, *Athene noctua* and *Strix aluco*) were recorded in the Park, at five different locations with elevations ranging from 615 m to 655 m above sea level. Only one of the three recorded owl species (*Strix aluco*) was previously reported by Roberts (1991) from the park whereas the other two owl species are being reported for the first time from the MHNP. In current study, we did not record three earlier on reported owl species (*Glaucidium cuculoides*, *Otus sunia* and *Otus brucei*). Nests of spotted owl (*Athene brama*) were recorded at Kalinger, Gandhian and Shah Allah Ditta sampling sites, those of Little Owl (*Athene noctua*) at Kalinger site while the single nest of the Tawny Owl (*Strix aluco*) was found located in a cavity on a mountain ridge at Kalinger sampling site. The major vegetation species present at the nesting sites of the owls included shrubs like Bhaikar (*Adhatoda vasica*), Kandiyara (*Echinopus echinatus*), and trees including kikar (*Acacia arabica*),



Phulai (*Acacia modesta*), Taman (*Grewia tenax*), Beri (*Zizyphus jujuba*), Sumbal (*Bombax ceiba*), Mulberry (*Morus alba*), Shreen (*Albizia lebbeck*), Grinda (*Carissa opaca*) and Bohr (*Ficus bengalensis*).

### CONSERVATION STATUS OF MUSK DEER (*MOSCHUSCHRYSOASTER*) IN PAKISTAN-REVIEW PAPER

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Pakistan geographic location, climate conditions and resource availability accounts positive credit that make this area rich with biodiversity. Many species migrate towards Pakistan in search of food, shelter and protection. Musk deer is one of those species that like to stay in Himalay region, native species of sub-alpine scrub and coniferous tress area including Machira, Neelum valley of AJK, Indus Kohistan, Chitral, Astore, Chilas, and Gilgit. The specie was declared endangered and was listed under Appendix I of CITES. The population of Himalyan musk deer is declining due to its illegal hunting, hunting for musk pods, habitat degradation. The population of musk deer's was assessed by using camera, e-tagging and by counting. In 1998-1999 population of musk deer was recorded 2-3 animal's km<sup>-2</sup> which was decreased in 2004 and species reached to 35. Conservation efforts in 2006 increased musk population to 64 in Machiara. Conservation efforts include formation of Salkhala Wildlife Sanctuary (AJ&K), Drosh ,machiara National Park, AJ&K. Projects by Rufford Small Grants Foundation launch awareness campaign as well as count habitat and population distribution of the specie. The paper will be helpful in determining the conservation status of Musk deer in Pakistan and will help in devising better conservation for preventing the extinction of musk deer species.

### A REVIEW OF THE CONSERVATION STATUS OF ASTOR MARKHOR

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The Astor Markhor or the Flare-horned Markhor (*Capra falconeri falconeri*) is an endangered species adapted only to mountainous terrain in the Nothern part of Pakistan. It is a beautiful wild animal with twisted horns and name derived in reference to its ability to kill snakes. The Markhor - the national animal of Pakistan- is a keystone species and their presence keeps the food web in shape both for nature and humans. The species is severely fragmented with less than 250 mature individuals and a continuing decline of 20% estimated in 2 generations. The review highlights the threats surrounding Astor Markhor occurrence, issues regarding their conservation actions and the discussion on the role of organizations, local communities and international programs, benefits and ineffectiveness in sustainable management of this wildlife. The information has been gathered from research papers, newspaper articles, organizations, online sources and unpublished reports. The paper demonstrates that creating awareness, enforcing laws, protecting the habitat, limiting translocation and dismissing the ownership of local communities assert the best practice of conserving the dwindling animal.

### NESTING USE OF PLANT SPECIES BY AVIFAUNA INHABITING PABBI RANGE, KHARIAN

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The current study was carried out in the Pabbi Range Forest Kharian (32.811°N and 73.865°E), District Gujrat from September 2013 to June 2015 to investigate the nesting use of plant species present in the area by the avifauna. Five sampling sites were selected on the basis of presence of birds' nests and data were recorded by direct field observations. A total of N= 33 active nests of various birds species were recorded at five selected sampling sites; the nests of common myna (*Acridotheris tristis*), house crow (*Corvus splendens*) and Indian robin (*Saxicoloides fulicatus*) were found built on kikar (*Acacia nilotica*) and safaida (*Eucalyptus camaldulensis*), black drongo (*Dicrurus macrocerus*) also used *Eucalyptus camaldulensis*, while black kite (*Milvus migrans*) utilized simal tree (*Bombax ceiba*) for its nesting. The weaver bird (*Ploceus philippinus*) used *Acacia nilotica* for nesting, while at one sampling site (site-V), a colony of nests of house crow were found located on poplar tree (*Populus deltoides*). The bird species that showed breeding activity included house crow, common myna, black kite, black drongo, red turtle dove, and Indian robin, for which the clutch size and hatching success were recorded. Results of current study provide baseline information about vegetative preference of various bird species for nesting as well material composition, height and elevation of the nests. The records of nests of six different bird species in the study area are indicative of their successful breeding in the Pabbi range Kharian.

### HABITAT PREFERENCES OF WILD MAMMALIAN SPECIES ALONG RIVER CHENAB, PAKISTAN

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Present research was conducted to assess the habitat preferences of mammalian species along river Chenab, Pakistan. The mammalian diversity was recorded along forested landscapes, cultivated plantations, semi-urban and urban areas through direct and indirect observations. The habitat preferences of large, medium and small mammals varied significantly. A decline in mammalian diversity was observed from forest habitat to urban landscapes. Indian wild boar, Asiatic jackal, Indian fox, jungle cat, Indian pangolin and long eared desert hedgehog preferred forested areas as well as slightly modified habitats while Northern palm squirrel, house mouse, house shrew and rat species preferred human habitations. Similarly, few species like small Indian mongoose, Soft-furred field rat, short tailed mole rat, Asiatic jackal and Indian gerbil preferred cultivated areas. It can be concluded from present study that many of the mammalian species are habitat specific and corridors and connections between different landscapes are important for the conservation of mammalian diversity.

**COMPARATIVE STUDIES ON GROWTH PERFORMANCE, CARCASS YIELD AND SENSORY QUALITY ATTRIBUTES OF MEAT BETWEEN TURKEYS (*MELEAGRIS GALLOPAVO*) REARED IN FREE-RANGE AND CONFINEMENT REARING SYSTEMS**

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Growth performance, carcass yield and sensory quality parameters of meat of turkeys *Meleagris gallopavo* reared under free-range and confinement rearing systems were compared. There were two treatments, each containing 25 birds. In indoor treatment, the turkeys were raised in a 20 × 20 feet (length × width) well ventilated room. In the free-range treatment, the birds were housed in an open cage having same dimensions *i.e.* 20 × 20 feet (length × width), in addition, they also had a free-range grass paddock. To compare sensory attributes 4 ready to-cook turkeys having equal size were selected from free-range and in door rearing systems. These birds were skinned and boneless breast fillets and boneless thighs were taken, weighed and steam roasted. The roast breast meat of free-range turkeys was darker and yellower whereas cooked thigh was light yellow than meat from indoor birds. Score for breast meat tenderness and appearance varied significantly ( $P < 0.05$ ) between free-range and confinement reared birds. However, non-significant differences were recorded for thigh meat tenderness of free-range and confined turkeys. Significantly higher ( $P < 0.05$ ) meat redness was recorded in thigh meat of turkeys reared in free-range system than thigh meat of confined *M. gallopavo*. While non-significant differences in breast meat color were observed between free-range and confined reared birds. Significantly higher ( $P < 0.05$ ) thigh and breast meat was observed in confined birds and there the juiciness and oiliness was higher in confined reared birds as compared to the free-range reared turkeys.

**PRELIMINARY FINDINGS OF BEHAVIORAL PATTERNS IN CAPTIVE CHINKARA (*GAZELLA BENNETTII*) AND PROSPECTS FOR FUTURE CONSERVATION**

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Understanding behavioral patterns plays a vital part in determining appropriate management systems. Present study was planned to determine the effect of captivity on behavioral patterns of Chinkara *Gazella bennettii*. The time spent in different behavioral activities viz. resting, standing-alert, locomotion, feeding, drinking, ruminating, tail-pasting, urinating/defecating, self-directed behavior, environmental sniffing, ano-genital sniffing, affiliative interaction and agonistic interaction of 10 wild-caught and 10 captive-bred adult Chinkara were recorded and compared through focal sampling for a period of six months. Non-significant variations were recorded among wild-caught and captive-bred animals for all behavioral activities. However, male *G. bennettii* showed relatively higher frequency for agonistic behavior. The results suggested that captivity has

no immediate impact on the behavioral patterns of captive Chinkara. However, further studies are needed for effectiveness of captivity and farming of *G. bennettii*.

### COMPARISON OF BEHAVIOR AND HABITS OF INDIAN BLUE PEAFOWL (*PAVO CRISTATUS*) IN FREE-RANGE LIVING AND CAPTIVITY

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The aim of this study was to observe the behavior and habitat of Indian blue peafowl *Pavo cristatus* in both the wild and captivity to see either the behavior is similar or different from each other. Observation showed the major and minor differences in roosting, nesting, feeding habit, social, reproductive and mating behavior and also some other behaviors were observed mainly in the captive peafowl. *P. cristatus* has solitary and communal roosting behavior and roosts on tall trees, females roost on the ground with chicks (Yasmin 1994; Harikrishnan *et al.* 2010). Peafowl are omnivorous and feed on insects, reptiles, grains, seeds (Yasmin *et al.* 1996; Ali, 1979; Johnsingh and Murali, 1978, ). Peafowl has a lek like mating system (Hillgarh and Rands *et al.* 1984, Loyau *et al.* 2007). The train feather of the peacock is the obvious visual display to attract females for the mating during breeding season (Gadagkar, 2003; Harikrishnan *et al.* 2010). The observation of the captive peafowl was carried out at the Zoological Garden, Nishtar Road, Sir A.K. III road, Karachi, Sindh, Pakistan from January 2015 to December 2015. Weight and height was measured with the different measurement instruments and weight machine and direct observation was done. Photographs and videos were taken to observe the behavior with respect to their roosting, feeding, breeding, nesting, hatching, parental care, mating, dust bathing and other social behaviors. Hatchability, survivability and mortality of chicks were calculated during the whole breeding and mating season of 2015 in Zoological garden.

### YEAR AROUND AVIAN DIVERSITY OF SAFARI ZOO LAHORE, LAHORE, PAKISTAN

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The study was conducted to observe the yearly round bird's species diversity from October 2014 to September 2015 at Safari Zoo Lahore. Safari Zoo Lahore is located in (31° 22' 57" N, 74° 12' 47" E and elevation is 208m) district Lahore, Punjab, Pakistan. The objective of the study was to measure avian fauna as an indicator of healthy environment along with main threats to avian fauna at study area. In total, 5456 number of bird's individuals belonging to 71 species covering 39 families and 14 orders were recorded. The total bird's species distribution was; year around residents were 51 (71.8%), winter migrators 12 (16.9%), and 6 (8.45%) summer breeder. The maximum avian fauna was observed in winter season as compared to summer. Among most dominant species were house crow *Corvus splendens* (n=2518), house sparrow *Passer domesticus*

(n=772), common myna *acridotheres tristis* (n=327), black crowned night heron *nycticorax nycticorax* (n=190) and jungle babbler *turdoides striatus* (n=186). Among 71 species only one species, alexandrine parakeet, was near threatened according to IUCN status. Population pressure, habitat degradation and unawareness are the main threats that ultimately will cause reduction in species number around the study area. Therefore, there is an urgent need to protect avian fauna by maintaining natural habitat of the area.

#### BIODIVERSITY OF LAL SUHANRA NATIONAL PARK IN WINTER SEASON

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The field surveys of Lal Suhanra National Park Bahawalpur were conducted to measure its biodiversity between October 2015 and December 2015. The study was commenced to determine the status of fauna and flora of park, diversity indices and environmental intimidations to their protection. The data was collected by point count method. Flora of park included 147 species of 14 families of plants. We have observed 20 species of mammals while four of them were threatened. 11 species of amphibians and 21 species of reptiles were found there. In amphibians 2 species were vulnerable and 1 was endangered. Results of the study showed that maximum account of 7443 birds of 74 species that belong to 35 families and representative of 16 orders were witnessed during October 2015 to December 2015. The Maximum values of diversity indices were also documented in study year 2015 i.e. Shannon-Weiner Diversity Index (H') was 4.18; Census Index (CI) was 8.5/Km<sup>2</sup> and Simpson's Diversity Index (D) was 0.98. According to status of bird's existence status for the Lal Suhanra, 70 were very common, 3 were common, fairly common was only 1 and no rare species was found. The season wise distribution of bird's species shown that year around resident were 76%, 7% were summer breeders, 4% were passage migrants while 5% were uncommon and 8% were common winter migrants. The most dominant species of study area were *Corvus splendens* (n=425), *Passer domesticus* (n=325), *Elanus axillaries* (n=245), *Acridotheres tristis* (n=185) *Pycnonotus leucotis* (n=145). Hunting, deforestation, wood logging, disturbance by human activities and habitat deforestation are major threats for wild life animals at Lal Suhanra. Reforming of natural lakes for recreational purposes is also major threat for birds and animals. Proper attention should be given to existing habitat of study area particularly during the course of migratory birds.

#### ENRICHMENT TOOLS TO TACKLE STEREOTYPIC BEHAVIOUR OF BEARS IN BALKSAR BEAR SANCTUARY, PAKISTAN

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Most of the bears when kept in semi captive or captive environment show stereotypic behavior. However, introducing enrichment structures cause decrease in stereotypy. Current study

was conducted in Balksar Bear Sanctuary to study the behavioral changes before and after introducing enrichment tools in 8 quarantine bears *i.e.* Asiatic black bears (*Ursus thibetanus*) and 17 enclosure bears which include 3 Himalayan brown bear (*U. arctos*) and 14 black Asiatic bears (*Ursus thibetanus*). Ethograms were made for active, passive and abnormal behaviors for focal and scan sampling of bears. Wooden logs, cartons and dry grass were used as enrichment structures. Results showed positive behavior of bears in enclosure even without enrichments. There was increase in active behavior of bears both quarantine and enclosure after enrichment structures were introduced. Stereotypy was also reduced. Therefore, application of enrichment tools may bring long term changes in behavior of semi captive bears.

#### STUDIES ON AVIFAUNA DIVERSITY OF JALLO WILDLIFE PARK, LAHORE

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Studies on avifauna diversity was conducted at Jallo Wildlife Park, Jallo Village, Lahore from January through August, 2015. The Jallo Wildlife Park covers total area of 461 acres. The area was divided into five blocks and direct as well as indirect techniques of population estimation were used to record the avian diversity of the park. Monthly surveys were conducted during dawn and dusk hours and a total of 65 bird representing 42 genera and 33 families were recorded from the study area. Out of these 65 species, 44 species were resident, 11 species were winter visitors while 2 species were recorded as summer visitors.

#### NEW DISTRIBUTION RECORDS OF SMOOTH-COATED OTTER (*LUTROGALE PERSPICILLATA*) FROM BALOCHISTAN, PAKISTAN

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According to the review of literature, Smooth-coated otter (*Lutrogale perspicillata*) has never been reported from the Balochistan Province of Pakistan. But a few reports from some fish farmers and hunters in Balochistan and personal communications with the officials of Balochistan Forests & Wildlife Department about the existence of the species in some of the areas of Balochistan adjacent to Sindh Province provided the base for investigating the existence of the species in Balochistan. The study was conducted by a team of 11 members from November 10-19, 2015 under an HEC funded project. The main objective of the study was to record the existence of Smooth-coated otter (*Lutrogale perspicillata*) in Balochistan province of Pakistan. Prior to the field visits, a number of people including local field biologists, teachers, fish farmers, hunters and officials of Balochistan Forests & Wildlife Department were contacted and interviewed using a pre-

developed questionnaire. Based on the information gathered through interviews and personal communications with the officials of Balochistan Forests & Wildlife Department, 15 different sites were marked on the map of Balochistan Province where, according to the persons interviewed, the otter was present. The 15 identified sites were then physically visited to confirm the existence of the species and different direct and indirect methods including direct observation, observing tracks, Holts, spraints and feeding remains of the species were applied. Around 2,000 km were traversed in Balochistan covering 15 different sites in five districts and the existence of Smooth-coated otter was confirmed at three sites in three different districts. The species was found in isolated and fragmented habitats and according to the local residents, its appearance is recently noticed especially after the heavy rains and floods in Sindh and Balochistan during 2010 and 2011.

#### EVALUATION OF SINGLE SLIDE FECAL SAMPLES OF ZOO ANIMALS THROUGH MICRO FLOATATION TECHNIQUE

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Fecal samples of zoo animals are evaluated for the detection of adult parasites, larvae, ova or the segments of the endo-parasites. All relevant techniques require a considerable amount of fecal material obtained from the animals. If, in any case, there is less amount of fecal sample question arises how it can be evaluated. To evaluate a minute amount of fecal sample as low as 0.5-1.0 gram can be examined by a newly developed technique "Micro- Floatation Technique". This technique proved very much effective in qualitative and quantitative study of ova, oocyst and eggs of internal parasites. The samples obtained from zoological garden of Dera Ghazi Khan, Punjab, Pakistan were evaluated by "Micro-floatation Technique". The fecal sample of zoo animals included Black buck (*Antelope cervicapra*), Deer (*Artiodactyl cervidae*), Cheetal (*Axis axis*), Chinkara (*Gazella gazelle*), Emerald (*Amazilia brevirostris*), Neel gay (*Boselaphus tragocamelus*), Mufflin sheep (*Ovis aries orientalis*), Wolf (*Canis lupis*), Lion (*Felidae panther leo*), Goose (*Anser indicus*), Zeebra (*Equidae burcheli*) and Emu (*Dromiceius novahollandiae*) showed a low infestation of oocysts, cryptosporidium cyst, eggs of strongyles and nematodaris.

#### VARIATIONS IN SERUM CHEMISTRY IN MALE AND FEMALE RING-NECKED PHEASANTS (PHASIANUS COLCHICUS) IN CAPTIVITY

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Variations in hematological parameters for adult male and female ring-necked pheasants (*Phasianus colchicus*) were recorded. Forty adult ring-necked pheasants (20 ♂, 20 ♀) were kept in separate cages, each cage having separate drinking and feeding facilities. Five birds per cage were

confined and these cages were housed in a well-ventilated 20 × 20 feet (length × width) room. Blood samples were taken from ulnar vein. Different blood and serum chemistry parameters such as red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb ) concentrations, mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), heterophils, lymphocytes, monocytes, eosinophils, basophils, ALP, uric acid, cholesterol, total serum protein, albumin and creatinine were determined among adult male and female pheasants. Non-significant differences in RBCs, WBCs, heterophils, eosinophils, MCHC and Hb values were observed among male and female pheasants. Significantly, higher values of lymphocytes, monocytes and PCV were observed in males while higher basophil count was observed in female as compared to male birds. Significantly higher values for ALP, cholesterol, total serum protein and creatinine were observed in males while higher uric acid values were observed in females as compared to male *P. colchicus*. However, non-significant differences in albumin were recorded among male and female birds.

#### MEGA-WILDLIFE DIVERSITY OF CHOLISTAN GAME RESERVE AND THEIR ECOLOGICAL IMPORTANCE

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The present study was conducted to enlist all observed and identified wildlife diversity at Cholistan Game Reserve. The objective of the study was to investigate the existing wildlife diversity status, density and their ecological importance. Data were collected by direct count methods. The mega-wildlife diversity identified includes 67 species of birds, 22 species of birds, 09 species of mammals and one species of amphibian. Birds density recorded was 0.00166 birds/hectare. Shannon Weiner diversity index (H') value encountered birds was 2.453. The most dominant species of birds was Desert Wheatear *Oenanthe deserti* (31.2). In reptiles the dominant species recorded was Indian Fringed-toed Sand Lizard (*Acanthodactylus cantoris*) whereas in mammals the dominant species recorded was Chinkara (*Gazella bennettii*). Maximum chinkara *Axis porcinus* population was observed in May and Houbara bustard in December to January. Wildlife diversity benefits humans by providing important ecosystem services which include Houbara bustard and Chinkara were providing game meat for food. Scavengers were regulating ecosystems by scavenging carcasses and waste. Predators (carnivorous species 43%, insectivorous 19%) were controlling populations of invertebrate and vertebrate pests. The granivorous species (15%) were regulating pollination and seed dispersal of plants. Seasonal occurrence of birds species observed revealed that 43.3% year round resident, 40.3% winter migrant, 10.4% passage migrant and 06% summer breeder. The conservation status of Sand Cat (*Felis margarita scheffeli*) and Desert Fox (*Vulpes vulpes pusilla*) were recorded as near threatened. The ecological functions provided by wildlife diversity in the study area was game meat for food, animal excreta for fertilizer, regulating ecosystems by scavenging carcasses, controlling populations of invertebrate and vertebrate pests, supporting services by cycling nutrients and by contributing to soil formation. The mag wildlife diversity is ecologically important; therefore, their conservation is needed.



**A STUDY OF THE BEHAVIOUR OF CAPTIVE OSTRICHES (*STRUTHIO CAMELUS*) AT  
ZOOLOGICAL GARDEN, KARACHI AND ITS COMPARISON WITH OSTRICHES  
LIVE IN WILD**

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This paper describes the behaviour of ostriches (*Struthio camelus*) were in captivity, where it shows some different requirements and capabilities as compared to the wild specimens. The behaviour was observed in three categories, *i.e.*, reproductive behaviour (dancing, fighting & mating), mobility behaviour (standing, walking, sitting, running & sand-bath) and nutritional behaviour (feeding, drinking, pecking, urination-defecation & coprophagy). Ostriches show similar behaviour as compared to the specimens survive in the other captive environments, a few differences were observed. The *S. camelus* reared in captivity posed similar behavioural patterns to those remaining in the wild, although some captive circumstances imposed minor differences (Hambaliet *et al.*, 2015). It is the largest, flightless, herbivorous bird, found in open range habitat types and is endemic to Africa (Brown *et al.*, 1982). The mean daylight overall time budget for nutritional behaviour was 35.66% for females and 29.92% for males, whereas the mean daylight overall time budget for reproductive behaviour was 8.88% for females and 13.11% for males (Obeid *et al.*, 2012). The most prevalent noted behaviour was thermoregulation followed by pecking, twirling and aggression respectively (Mushi *et al.*, 2008). The behavioral observation of an ostrich *S. camelus* was studied at Karachi Zoological Garden, Nishtar Road, Sir A.K. III road, Karachi, Sindh, from January 2015 to December 2015. The adult specimens were of same age with their chicks. Their height and weight was measured with different intervals. Their feeding compositions, day and night behavior, interaction with each other and with human were also observed. Their roosting and feeding behavior was also observed. Photographs and videos were taken to study their different behaviours.

**FATAL AND NONFATAL ATTACKS BY ASIATIC BLACK BEARS (*URSUS  
TIBETANUS*) IN DISTRICT MANSEHRA, PAKISTAN: 2001-2015**

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Asiatic black bear (*Ursus thibetanus*) is one the most powerful, robust and agile wild animals and if they feel threatened they defend themselves, their young ones and territories as well. Human-bear conflicts are common in bear-dominant areas and particularly when humans are intervening in their habitat due to fast human population growth. In Mansehra district, species is found in Siran and Kaghan Forest Divisions and at least 25 people were exposed to bear attacks resulting 6 fatal and 19 nonfatal injuries during 2001-2015. Fatal bear attacks occurred in the Siran Forest Division SFD (n= 4) and in the Kaghan Forest Division KFD (n= 2). Fatal attacks in the SFD were 2 times as many compared to the KFD. Of fatal attacks, 50% (3 of 6) occurred in 2005, 16.6% (1 of 6) in 2006. 16.6% (1 of 6) in 2010 and 33.3% (2 of 6) in 2015. All fatal attacks were occurred on single

person and made by female bears. Likewise, 19 people were injured by Asiatic black bears and injuries occurred in SFD (n=8), while in KFD (n=11). Date and timing of attack, number of bears, behavior of bear during attack, place of incident, number of victims, age and gender of victims, and activity of victims during attack are also brought under considerations. At least 14 Asiatic black bears were retaliatory killed in this time period as well. Killed bears in the SFD (n=5) and in the KFD (n=9). Our research suggests that concerned departments, by only managing Asiatic black bears and by interacting with local communities can understand the human bear-conflict. With proper training and mass awareness programs at local level, locals can learn to recognize the behavior of species, and their ecological importance as well and are the best tools to mitigate human-bear conflict.

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#### **CULTURAL AND MEDICINAL SIGNIFICANCE OF AVIAN FAUNA ALONG THE RIVER CHENAB, PUNJAB, PAKISTAN**

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This research paper provides information on cultural significance of avian fauna of river Chenab, Pakistan. This is first ethno-ornithological study from the study area in which popularity level of avian species is computed through Relative Popularity Level and Rank Order Priority. One hundred nine interviews were applied to compile socio-economic information, qualitative data on cultural significance from three selected districts in the territory of the river Chenab. The compiled data are analyzed using different quantitative tools, such as relative frequency of mention (RFM), fidelity level (FL), relative popularity level RPL and rank order priority (ROP). A total of 155 avian species with cultural uses are compiled. The highest RFM was recorded as 0.58 in House Sparrow (*Passer domesticus*). The highest FL (100%) was recorded in House Sparrow (*P. domesticus*) and Domestic chicken (*Gallus gallus*). The value of RPL and ROP were compiled to recognize the folk avian species wealth of this area. Ten species were most popular, while remaining species were uncommon in the study area. The ethno-ornithology results of current study showed that the rich diversity of avian species play an important role for the cultural and health care of native communities. The avian species with highest RPL value should be screened for nano-particle, molecular and pharmaceutical analysis.

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#### **CHARACTERIZATION OF MALE ASIAN KOEL (*EUDYNAMYS SCOLOPACEA*) VOCALIZATION**

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Bird vocalization is the most efficient way of communication and is unique to avian species. This is so because, evolutionarily, the olfactory system of birds is relatively less well developed. Compared to song birds the vocalization in non-passerine birds has been given less attention. The present study was an attempt to describe the vocalization of male Asian Koel on the basis of

spectrogram analyses and associated behavior. Recordings were carried out in three different research sites by using a directional microphone connected to the digital recorder. The visual inspection of spectrograms and waveforms of vocalization and the associated behavior in the field were used in the present study for identification of different call types. A total of six different types of calls were categorized. The “melodious call” consisted of a basic primary syllable, which makes an average of eight to twelve secondary syllables as compared to melodious calls. The secondary syllables contributed to form aggregates in aggressive calls, while in case of low aggressive calls, both the differentiated and undifferentiated secondary syllables were present. The “very short duration calls” in which the primary syllable produced no secondary syllables but occasionally produced 2-3 short length secondary syllables were categorized as “territorial calls”. It was further revealed that koel birds also emitted short and less spontaneous “calling calls” in a continuous manner. The starting calling calls were of shorter length as compared to the middle or end calling calls. All types of calls were statistically different ( $P < 0.05$ ) from each other as regards acoustics parameters. Future studies are required to investigate detailed communication behavior in non-passerine birds.

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**HABITAT PREFERENCES AND BREEDING PARAMETERS OF HOUSE SPARROW  
(*PASSER DOMESTICUS*) IN URBAN COLONIES OF RAWALPINDI**

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House sparrow (*Passer domesticus*) was once thought as the most acquainted species in human habitations, now facing serious population decline in many regions of the world. Beside many other reasons, it is also presumed that degree of urbanization might be negatively influencing the population of house sparrow. Current study encompasses the aspects related to breeding biology and habitat preference of house sparrow in varyingly urbanized areas of Rawalpindi, Pakistan. Observations from three different zones (five sites): 2 metropolitan, 2 residential and 1 parkland, were recorded during January to July 2015. In addition, breeding parameters were also assessed by installing 20 nest boxes in different locations. A total of 395 nest were recorded during the study period, which were specifically 210, 195 and 20 nest from residential, metropolitan and parkland area respectively. Nesting frequency was highest in month of May while it was lowest in January suggesting its peak breeding activities during mid-summer months. Out of 20 installed nest boxes, 14 were occupied by house sparrow with a mean (Minimum 4 & maximum 6) of 4.8 (average) eggs per nest. Hatching period of about 11 days has been found during this study. We concluded that this species still prefer human habitations with older building structure which facilitate its nesting, breeding and feeding. Further, it is suspected that, in addition to other biological and ecological factors, loss of proper nesting locations and feeding sources in modern building structure, might be contributing towards lessening the population of house sparrow in more urbanized regions. It is suggested to periodically assess the population trends (at least biannually) and potential threats to this species to avoid further population decline in future.

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**FOOD ANALYSIS OF *FALCO CHICQUERA* 'RED NECKED MERLIN' (FALCONIDAE) AND *BUTEO BUTEO* 'COMMON BUZZARD' (ACCIPITRIDAE) FROM SOUTHERN PUNJAB, PAKISTAN**

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The prey preferences of Red necked merlin *Falco chicquera* occupying Lal Sohanra National Park and common buzzard *Buteo buteo* at Taunsa Barrage, southern Punjab were analyzed. The merlins were more diverse (euryphagous) in feeding habits with maximum feeding during winter and spring while buzzards were less diverse (stenophagous) in feeding with feeding at its peak during winter, spring and summer. Out of 500 pellets collected from 04 nests of Red necked merlin 750 prey items were recovered. The merlin preferred birds in their diet (390) followed by small mammals (120), rodents (75), frogs/toads (70), insects (45), lizards (30) and bats (20). Whereas, 400 pellets collected from 03 nests of buzzard, 525 preys were identified. The buzzard preferred small mammals in their diet (167) followed by insects (136), chicks (122), lizards (72) and worms (28).

**INTRA AND INTER-SPECIES VARIATIONS IN MORPHOMETRIC MEASUREMENTS OF THREE STRUNIDS; COMMON STARLING (*STURNUS VULGARIS*), JUNGLE MYNA (*ACRIDOTHERES FUSCUS*) AND PIED MYNA (*GRACUPICA CONTRA*) FROM DISTRICT MIANWALI, PUNJAB, PAKISTAN**

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Morphometric information of birds has largely been used to trace morphological and physiological changes which occur with the course of evolutionary history of a species. Moreover, morphometric information gives an insight about the parameters associated with intra- and interspecies variation which is a prerequisite to understand population dynamics. Regional variation in morphometric measures allows to comprehend the extent of adaptation as well as migration of a species. Present study was carried out to investigate intra- and interspecies differences in morphometric measures of three species of strunidae family. For this purpose, morphometric data of 73 adult specimens: 41 common starling (*Sturnus vulgaris*), 18 jungle myna (*Acridotheres fuscus*) and 14 pied myna (*Gracupica contra*) was obtained from Mianwali District, Punjab,

Pakistan. We measured body weight, total length, bill size, wing span, tail length and tarsus length for each individual. The calculated means (ranges) for morphometric measures of common starling, jungle myna and pied myna were: 63.80 (72-58), 73.16 (80-63), 61.85 (70-55) for body weight (g); 22.15 (23-20), 23.33 (24-22.5), 22.70 (23.4-22) for total length (cm); 2.70 (3-2.5), 1.97 (2.25-1.5), 3.00 (3-3) for bill length (cm); 36.12 (37.5-35), 36.22 (37.5-35), 36.50 (37-35) for wing span (cm); 6.34 (6.8-6), 7.65 (8.5-7), 6.82 (7-6.5) for tail length (cm) and 5.55 (6.5-6), 6.69 (7-6), 6.82 (7-6.5) for tarsus length including foot (cm), respectively. We observed a significant variation of body weight, bill, tail and tarsus lengths among species. Besides these we found certain noteworthy intra-species variations for some of the morphometric measures. We predicted that a combination of genetical, biological and ecological factors are synergistically influencing for this observable intra and interspecies morphometric variations.

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**ABUNDANCE AND HABITAT OF GREY PARTRIDGE (*FRANCOLINUS PONDICERIANUS*) IN DAKHN AIR FOREST, DISTRICT ATTOCK**

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The present study on Grey partridge (*Francolinus pondicerianus*) was conducted in Dakhnair forest, district Attock from March 2014 to February 2015 with the objectives to determine its population density and habitat status. The study area was stratified into three different habitat types viz., cultivated fields, natural forest and open areas. Data on population density was collected through direct observations using line transect method, laying two transects in each habitat type, each 200 m long and 50 m wide. To determine the habitat use, vegetation surveys were conducted in its habitat taking 60 quadrates in the study area. Relative density, relative frequency, relative cover and Importance Value Index were calculated for all plant species recorded from the habitat of Grey partridge. Overall population density of Grey partridge in the study area was  $3.66 \pm 0.58/\text{km}^2$ . Cultivated area had highest density of  $1.83 \pm 0.44$  partridges/ $\text{km}^2$  while forest and open area habitat had  $1.08 \pm 0.22$  and  $0.75 \pm 0.21$  partridges/ $\text{km}^2$ . Average population densities were not significantly different among habitats as judged by one-way ANOVA ( $P=0.05$ , d.f=2 at  $\alpha=0.05$ ). Collectively, 23 plant species were recorded from three habitat types in the study area out of which 4 were trees, 4 shrubs, 10 herbs and 5 grasses. Major tree species included *Ziziphus jujuba*, *Acacia nilotica*, *Acacia modesta* and *Dalbergia sissoo*. Dominant shrubs species with higher importance value included *Sacchrum munja*, *Opuntia dillenii*, *Calotropis procera* and *Dodonea viscosa*. Whereas the dominant herbs species were *Rhazia stricta vina*, *Aerva javanica*, *Dacus carota*, *Oxalis cerniculata*, *Echinops echinatus*, *Parthenium hysterophorus*, *Carthamus oxyacantha*, *Chenopodium album*, *Verbascum thapsus* and *Brassica campestris*. The important grasses included *Desmostachya bipinnata*, *Cynodon dactylon*, *Aestivum setivum*, *Heteropogon contortus* and *Typhoidum pinnafidum*. Most preferred habitat type in study area by Grey partridge was cultivated area dominated by *Aestivum setivum* (IVI= 37.22) as compared to forest and open areas dominated by *Dalbergia sissoo* (IVI= 78.49) and *Ziziphus jujuba* (IVI= 70.34), respectively. Presence of water sources was a regular feature of forest habitat that is frequently visited by Grey partridge, in addition to slopes, ridges and other dissected plains. Slope gives an escape way to the Grey partridge when it is disturbed, it moved to the upper and lower slope. At some points of dissected plains partridge were seen while foraging there which was thickly vegetated area providing food and cover to them. Steps required for better management and conservation of Grey partridge may include; controlling illegal hunting through the implementation of Wildlife Protection Act,

addressing habitat degradation problem by controlling deforestation, livestock grazing and creating awareness to local people, checking land encroachment problem for housing schemes and commercial poultry farming and initiating public education and awareness campaign about the importance of wildlife / biodiversity and its conservation.

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#### PHYSICO-CHEMICAL CHARACTERISTICS AND STORAGE STABILITY OF PASTEURIZED GOAT MILK

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The popularity of functional foods has been tremendously increasing in the last few decades due to their potential health benefits. The focus of food industry on the development of functional foods has also steered a trend of consuming foods with increased health benefits. Therapeutic perspectives of goat milk over bovine milk are already well established. In this study, physicochemical characteristics and storage stability of pasteurized goat milk was determined over a storage period of 9 days at 4°C using cow milk as control. The concentration of oleic, linoleic and linolenic acid in pasteurized goat milk was 18.42%, 1.84% and 0.92%, CLA content of goat milk was 0.38% as compared to 0.13% in cow milk. Peroxide value, anisidine value and conjugated dienes of 9 days stored pasteurized goat milk was not different from the cow milk. These results suggested that physical-chemical and storage stability of pasteurized goat milk was not different from the cow milk.

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#### STUDIES ON GROWTH TRAITS AND BIOMETRIC TRAITS IN GREY PARTRIDGES PERDIX PERDIX FED WITH DIFFERENT DIETARY REGIMES

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Present study was planned to determine the effect of different dietary regimes on body mass and biometrical traits in grey partridges *Perdix perdix*. Twenty four birds (8♂ and 16♀) were arranged in two groups, each having four birds. Birds in group I were fed with poultry feed while group II birds were fed with millet. Each group was considered as a treatment and there were three replicates for each treatment. All the birds were kept in separate cages provided with separate feeding and drinking facilities. Significantly ( $P < 0.05$ ) higher weight gain  $231.37 \pm 6.28$  and beak length  $2.2 \pm 0.1$  was recorded in *P. perdix* fed with poultry feed while longer wing length  $7.87 \pm 0.29$  cm, tail length  $8.8 \pm 2.85$  cm and spur length  $0.43 \pm 0.13$  cm were noted in birds fed with millet. However, non-significant variations in wingspan, tarsus length and shank length were observed for the poultry and millet fed birds. Significantly, higher feed intake was observed in birds fed with millet than poultry feed. However, non-significant variations were recorded in meat quality parameters viz. color, flavor, juiciness, tenderness, oiliness and overall acceptability among poultry feed and millet fed *P. perdix*.

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### ECOLOGY AND FEEDING BIOLOGY OF FERAL PIGEON (*COLUMBA LIVIA*) IN SOME AREAS OF KARACHI, PAKISTAN

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The ecology of feral pigeon (*C. livia*) had very much neglected and very minimum work has been done on their ecology. They live in cervices and attracted by human building structure. The aim of this research was to study the population density, sex ratio, roosting areas, nesting areas, food and water resources in four different areas of Karachi with respect to season. Four study areas had selected including Sindh High Court Sadder, Aram Bagh, M. A. Jinnah Road and North Nazimabad Hyderi to carry out the research. To study population density the plotting method used to Census the individuals. Number of male and female observed on the basis of physical body appearance in each season. Population density observed per kilometer and result showed that the population density of feral pigeon (*C. livia*) was high in the beginning of spring (March) as well as in the beginning of autumn (September – October). While the lowest population had been observed in summer season (May – July), in Sindh High Court, Aram Bagh, North Nazimabad Hyderi and M. A. Jinnah road respectively. Population density also influenced by architectural features. The highest population had observed in the areas comprising on old and historical buildings while the lowest population size had observed in areas including modern architecture. Food quality show great variation in every season. In Sindh High Court Sadder, North Nazimabad Hyderi and M. A. Jinnah Road pigeons were feeding as granivores while in Aram Bagh feeding as omnivore.

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### COMPARISON OF FLORA AND FAUNA OF GLOBALLY IMPORTANT SALT RANGE WETLANDS COMPLEX OF PAKISTAN

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The wetlands are the key to Pakistan's economy and environment. Pakistan's permanent and ephemeral wetlands are globally significant in two ways: first, in terms of the intrinsic value of their indigenous biodiversity and secondly, as an acute example of the poverty/subsistence-use nexus that constitutes one of the most fundamental threats to biodiversity worldwide. The high global significance of Pakistan's wetlands is attributable to the diversity of species that they support. Study were conducted to find out flora and fauna of salt range wetland complex. The selected were Kallar Kahar, Khabbeki, Ucchali, Jahlar and Namal Lakes. Direct and indirect field observations were recorded on the basis of direct counts and specimen collection and information about flora and fauna were gathered from interviews and general discussions with the local community. The wide diversity of species were identified on the basis of identification guides, professional field experience and the animal's habitat and ecology. Spotting scope binoculars and still camera were used for counting and identification of the wide range of bird diversity. Fish species were captured from each lake through local netting techniques for comparison. Physical and

chemical parameters of water of salt range wetland complex were recorded and analyzed in the laboratory. The results showed that the average mean temperature calculated in all the lakes were in the range of  $26 \pm 2.943$  to  $34.66 \pm 2.516$ , mean dissolved oxygen concentration in water  $3.46 \pm 0.808$ , to  $4.63 \pm 0.642$ , respectively. In the present study Mean salinity measured at Kabbaki, Jalhar and Uchalli were  $1100 \pm 100$ ,  $3500 \pm 200$  and  $24100 \pm 608.27$  while salinity measured at Kallar Kahar and Namal was  $1733.33 \pm 115.47$  and  $344.33 \pm 65.850$ . Five heavy metals *i.e.* (Cd, Cr, Cu, pb and Ni) were detected in water were in the range of 0.05 to 1.04 ppm in all the salt range wetland complex. 15 species of trees and 9 shrubs were recorded from different salt range wetland complex. The most commonly occurring plant species was *Acacia modesta* in the whole region. Total of 39 birds species were recorded from salt range wetland complex. Some of them were resident (R), wintering (W), irregular year round visitor (I), ordinary migrant (OM), vagrant (V), summer migrant (SM), passage migrant (PM). According to our study 17 small mammals' species were observed. The small mammals constituting rodents, insectivores, bats, mongooses, and the hedgehogs not only maintain ecological balance in a an ecosystem but also play a specific role in biological control necessary for a self sustained ecosystem.

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#### ECOLOGICAL ASSESSMENT OF NARMADA RIVER WITH SPECIAL REFERENCE TO DIVERSITY OF MOLLUSCANS

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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 f 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 throughout the study period. Highest Shannon and Weaver index (H) was observed in station II and lowest in station I. The minimum H value was observed in December and maximum in March. The result of the present study emphasizes the importance of conserving the world's freshwater molluscan population, which are declining at an alarming rate through habitat destruction and pollution.

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#### DIVERSITY OF EARTHWORMS (OLIGOCHAETA) IN BAHAWALPUR REGION

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Earthworm diversity was investigated in Bahawalpur area from October, 2014 through September 2015. Four habitat types viz. cultivated land in Adil town Abbasi, river bank, nursery and Baghdad campus The Islamia University of Bahawalpur designated as site "1", "2", "3" and "4" were sampled using hand sorting technique randomly from selected sites in order to explore the



diversity and distribution of different species of Earthworm both qualitatively and quantitatively. Qualitatively, a total of 1,211 specimens were recorded representing one family Megascolicidae, one genus *Pheretima* and three species *Pheretima posthuma*, *pheretima burliarensis* and *pheretima bournei*. *Pheretima posthuma* was the most dominant species among all the species found at the four study sites. *Pheretima posthuma* had the maximum mean density of  $116.33 \pm 10.50$  per  $m^2$  whereas *pheretima bournei* had the least density  $0.67 \pm 1.15$  per  $m^2$ . The maximum density was seen during the rainy season under the least disturbed green shady vegetation and minimum density was found during the winter season. The statistical analysis results showed that overall Earthworm diversity ( $1-D=0.374$ ) on the Simpson's scale was 37% and on the Shannon scale ( $H/\ln S E=0.624$ ) is 62.4%.

#### COMPARISON BETWEEN PARTHENOGENETIC AND NON-PARTHENOGENETIC SPECIES OF ODONTOBUTHUS (BUTHIDAE: SCORPIONS) FROM PAKISTAN

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In the present work the parthenogenetic and non parthenegenetic species of *Odontobuthus* were observed from different localities of Pakistan. The external morphology, genital components and chemical composition of their venom were also studied and these characters were compared with the representatives of family Buthidae recorded from Pakistan. Parthenogenesis with the exception of mites, is rare among Chelicerates (Palmer, 1991). It has, however, been observed in a few species of harvestmen, spiders and scorpions (Camacho 1994). Among 1600 species of scorpions, distributed throughout the world, eleven species were observed as parthenogenetic species (Lourenço, 1994). Lourenco (2000) discussed the different aspects of sexual reproduction and developmental stages of scorpions and also described phenomenon of parthenogenesis in their different species. Scorpion and its organs are used for the treatment of epilepsy, rheumatism and male impotency since the medieval period (Zargan *et al.* 2011). The juveniles of *O. odonturus* species were collected from various localities of Pakistan and were examined carefully. After their adolescence, males and females were placed separately to study their parthenogenetic characters. The morphological features and the identification features of specimens were also studied. Photographs of different organs of specimens were taken by Nikon L 320 (12 megapixels). Venom was extracted by electrically stimulating the base of scorpion's telson with commercial electric adapter was adjusted to 15 mVolt. Extrected venom was chemically analysed.

#### STUDY OF AMPHIBIAN DIVERSITY AND WATER QUALITY ASSESSMENT OF THEIR HABITATS IN SINDH, PAKISTAN

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Amphibians are sensitive to contaminants of their aquatic habitats, for that reason an extensive research was proposed to record diversity and physico-chemical parameters in ponds where amphibians dwelled permanently. Present study was conducted in some of the main areas of Sindh province such as Larkana, Shikarpur, Jamshoro, Hyderabad and Kashmore Districts from

January-December for the period of one year 2013. Six amphibian habitats were selected from each area from where amphibian diversity was confirmed using morpho-metrics and taxonomic literature. Water samples were collected by following the instructions of EPA and water samples were stored in well stopper polyethylene plastic bottles. Analytical apparatus used during the analysis included pH meter (Model: Orion, 420), conductivity meter (Model: Orion, 115), ultraviolet spectrophotometer (Model: Hitachi 200) and atomic absorption spectrophotometer (Model: Perkin Elemer Analyst 800), while some parameters were evaluated via titration procedures. Water quality was identified using water quality criteria of EPA (Environmental Protection Agency) and other concerned scientific literature. Altogether Three amphibian species *i.e.* *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis* and *Bufo stomaticus* were recorded from Larkana, Shikarpur and Hyderabad Districts, however one more species *i.e.* *Allopa hazarensis* was discovered from District Jamshoro and Kashmore. Water quality of all ponds was unsuitable for amphibian fauna as value of parameters was high. District Larkana was most polluted, while District Kashmore provided comparatively less contaminated habitats to them. Value of studied parameters varied every month, their elevated concentration was recorded in July, whereas most diminished value was noted in December but CO<sub>2</sub> varied contrary to other parameters. Water pollution may have negative effects on amphibians; therefore concerned authorities must take necessary attempts to save them in wild environment of Sindh Province.

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#### REDUCTION IN YELLOWNESS, GOATY FLAVOUR AND IMPROVEMENT IN VISCOSITY OF COW AND GOAT MILK BY BLENDING WITH BUFFALO MILK

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Although, cow milk is the most abundant source of milk on the globe; it is not the first choice milk in Pakistan because of its yellow color and low viscosity. Goaty flavor of goat milk is not acceptable to many people. This project has been undertaken with the objectives, to study the physico-chemical characteristics of blends of cow, goat and buffalo milk and to study the reduction in intensity of yellow color, goaty flavor and improvement in viscosity of cow and goat milk by blending with buffalo milk. Cow and goat milk were blended with buffalo milk from 15% to 45%, buffalo milk was used as control. Blends of cow, goat and buffalo milks were pasteurized at 65°C for 30 minutes, after cooling to 4°C, pasteurized milk samples were filled into 250 ml transparent PET bottles, stored at this temperature for 6 days. Milk composition peroxide value, TBA value, anisidine value, conjugated dienes and iodine value were determined at 0, 2, 4 and 6 days of storage period. Sensory evaluation was performed by a panel of ten semi-trained judges, milk samples were analyzed for color, flavor, smell and overall acceptability on a 9 point hedonic scale. Blending of cow and goat milk with buffalo milk increased the fat, protein content and viscosity. TBA value, anisidine value, conjugated dienes and iodine value of all the blends was not different from the control. A panel of ten trained judges was unable to detect goaty flavour in the blend containing 70% goat milk and 30% buffalo milk. Results of this investigation revealed that yellowness and goaty flavour from cow and goat can be decreased by blending with 30% buffalo milk.

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**EVALUATIONS OF CRUDE PROTEIN AND AMINO ACID CONTENTS FROM THE SCALES OF FOUR MULLET SPECIES (MUGILIDAE) COLLECTED FROM KARACHI FISH HARBOUR, PAKISTAN.**

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The main objective of this study was to determine the crude protein and amino acid contents in the scales of four mullet species found on Pakistan coast. These included *Liza macrolepis*, *Liza melinoptera*, *Mugil cephalus* and *Valamugil speigleri*. Crude protein content in dry matter of the scales of these mullet species ranging from 62.28 to 78.07%, which was found to be high in the scales of *L. melinoptera* and least in *V. speigleri*. The moisture content of scales ranged from 42.8 to 58.3%, high in *M. Cephalus* and low in *L. macrolepis*. Glycine, proline and arginine contents were found to be high in their scales, while cysteine, methionine and tryptophan were not detected. Thus, the result of the present study revealed that mullet scales can be consider as rich source of protein and amino acids.

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**EFFECT OF COMBINED FEEDING ON GROWTH, SURVIVAL AND BODY COMPOSITION OF SNAKEHEAD (*CHANNA MARULIUS*)**

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Studies were conducted to determine the effect of locally developed artificial feed on growth, survival, body composition and enzyme activity in digestive tract of yearling *Channa marulius*. There was single treatment and a control having two replicate ponds in each. Each pond has 250 fish. Fishes in treatment group received sole artificial locally pelleted feed containing 35% protein which was offered @ 1% body weight. Ponds in control group were regularly fertilized at every 4<sup>th</sup> day. Sampling was done after every fifteen days for weight and length measurements. Water quality parameters such as DO, salinity, electrical conductivity, pH, temperature, nitrates, phosphates and plankton productivity were regularly monitored. Control group has higher amount of copepods, ostracodes, cyclops and other phytoplankton than treatment group. Fish in control group gained significantly higher weight gain (87.2g), higher SGR(0.258), and FCR(0.5) than treatment group which lost weight (-22.2 g). There were significant variations in body composition too between two groups. Crude protein was higher, fat and ash were lower in control group

( $59.00 \pm 3.00^a$ ,  $9.00 \pm 2.00^a$ ,  $6.33 \pm 2.08^a$ ) when compared with that of treatment group ( $53.33 \pm 3.05^a$ ,  $7.33 \pm 2.08^a$ ,  $5.33 \pm 1.52^a$ ). In addition to these parameters moisture was higher ( $82.00 \pm 3.00^a$ ) in control group than treatment group ( $77.66 \pm 2.51^a$ ). Control group displayed higher lipase, protease, amylase activity ( $6.30 \pm 0.41^a$ ,  $6.82 \pm 0.20^a$ ,  $0.95 \pm 0.06^a$ ) than treatment group with artificial feed only ( $2.22 \pm 0.28^b$ ,  $3.53 \pm 0.43^b$ ,  $0.27 \pm 0.05^b$ ).

#### **ANALYSIS OF PHYSIO-CHEMICAL CHARACTERISTICS OF THE FRESHWATER RESERVOIRS FOUND IN PISHIN AND ZIARAT DISTRICTS OF BALOCHISTAN**

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The life on Earth is totally depends upon 'Water' which exist in nature in different forms such as river, ocean, clouds, lakes, snow and rain etc. Lakes are considered as most diverse, fertile, interactive ecosystem and productive in the world. Lake is usually describing different types of water bodies-natural, wetlands, manmade and ephemeral. On earth 3 % fresh available out of 0.01 % is only functional for the consumption of human. Water quantity and quality drops with decrease level of fresh water and inadequate supply of water on earth. Accordance to (WHO) World Health Organization, in developing countries 80% of illness and diseases are caused by water. Due to several pollutants, water is contaminated like several physico-chemical parameters such as pH, temperature, electric conductivity, transparency, hardness, phosphate, total nitrates etc. Physico-chemical and biological parameters have significant role in fitness of aquatic ecosystem. In aquatic organism strength, composition and dispersal physico-chemical parameters play key role. Present study is conducted on limnological study of fresh water bodies in order to determine the physical parameters such as temperature, pH, Electric conductivity, Total Dissolved Solids, turbidity and chemical parameters such as Dissolved Oxygen, Biological Oxygen Demand, Chemical Oxygen Demand, total hardness in both location Bostan and Spezandi dams. The main objective of the study is to determine the comparative pollution status and to determine heavy metals of Bostan and Spezandi dams. This study will be carried out at district Pishin and Ziarat Balochistan. The expected outcomes of research will to determent the water quality of district Pishin and Ziarat by different Physico-chemical characteristics and conservation technique to save water from deteriorate.

#### **AVIFAUNA OF BARA GALI SUMMER CAMPUS, UNIVERSITY OF PESHAWAR, KHYBER PAKHTUNKHWA**

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Survey of avian fauna of Bara Gali Summer Campus, University of Peshawar situated in Abbottabad was conducted from April to October, 2013. A total of 21 species belonging to 5 orders

and 15 families were recorded. Out of these, 6 were resident, 12 summer visitor and 3 rare. Order Passeriformes was represented by 16 species which are *Certhia himalayana*, *Megalaima virens*, *Phylloscopus trochiloides*, *Garrulax lineatus*, *Passer rutilans*, *Corvus macrorhynchos*, *Hypsipetes leucocephalus*, *Acridotheres tristis*, *Delichon dasypus cashmeriensis*, *Hirundo rustica*, *Muscicapa thalassina*, *Saxicola ferrea*, *Myiophoneus caeruleus*, *Parus melonolophus*, *Parus rufonuchalis*, *Parus monticolus*, belonging to 11 families. Two species *Dendrocopos himalayensis* and *Picus squamatus* belongs to only one family Picidae of order Piciformes. Among rest of the three orders each is represented by only a single species; Accipitriformes by *Accipiter virgatus*, Coraciformes by *Upupa epops* while order Psittaciformes has been represented by *Psittacula himalayana*. The distribution and abundance varied with season and maximum number of species were found during the monsoon season when most of the birds migrate for breeding. Some habits and behaviors like nesting, feeding, breeding and vocalizations were also studied which are very unique from other birds found on lower elevations. Among bird species adapted to diverse habitat in the field, Himalayan Jungle Crow, Common Mynas, Bulbuls, Barn Swallows, barbets were prominent. Interesting feature of the avian fauna is its familiarity with flora, was also observed during the present studies that some birds are very quick and active in their movement on a tree surface i.e. *Certhia himalayana*.

#### EFFECT OF DIFFERENT SALINITY ON BREEDING, FERTILIZATION, HATCHING AND SURVIVAL OF RED TILAPIA IN CAPTIVITY

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The present study was conducted to investigate the effect of different salinities with graded levels (0‰, 10‰, 20‰ and 30‰) on breeding of Red Tilapia for 60 days. Brood fish were stocked in hapa nets suspended in 4 cemented rectangular tanks (10x5x4 feet). 6 females and 2 males with average body weight 145g female and male 140g were stocked per tank and fed with commercial floating pellet having 35% crude protein with 2% body weight twice a day. Eggs were collected at 7 days intervals. The Results showed that highest eggs were obtained 1980, 1910 and 1890 on 0‰, 10‰ and 20‰ respectively and decreased quantity of eggs were obtained on 30‰ that were only 512 which is significantly different to each other ( $p \leq 0.05$ ). The maximum survival of fry achieved on 0‰ (1108), 10‰ (1070) and 20‰ (1058) while lowest survival achieved on 30‰ that is 75 numbers only which is also significant different from each other ( $p \leq 0.05$ ). Water quality parameters like as Temperature (28-29 °C), Dissolved Oxygen (6.2-5.9 mg/L), pH range (6.6-6.9 mg/L) and Ammonia less than 0.02-0.03 mg/L were monitored throughout the study period which are acceptable for this species rearing. Our results suggests that Red Tilapia can breed up to 20‰ Salinity and give maximum survival rate of fry.

**BIRD SPECIES DIVERSITY ON NATIVE FLORA OF SAFARI PARK**

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The present investigation deals with the population and avian diversity of safari park during the period from April 2015- December 2015. Total 36,179 individuals were recorded from the study area during recent study. Maximum density was observed in September 2015 and November 2015 favouring the food availability of food resources for birds. During research period total 22 species of birds were recorded belonging to 15 families. Minimum density of the avifauna was observed in August 2015 that is 3214 individuals. Avian diversity varied significantly as compared with the previous research conducted in 2013 and most of the species are extinct due to inadequate food availability, shelter resources and climatic changes. Rainfall rates were very low in year 2015 which effected the population rate of avian fauna of safari park. Safari park is a public amusement park due to which the pollution rate is increasing because of improper garbage dispersal system which heavily effects the environment and habitat of the birds. Many individuals belonging to family *psittacidae*, *alcidinidae*, *coracidae*, *campephagidae*, *motacilidae*, *turnidae*, *timaliinae*, *muscipapinae*, *falconidae*, *phasianidae*, *meropidae*, *alaudidae*, *oriolidae* were completely absent.

**SYSTEMATICS AND DISTRIBUTION OF LIZARDS *LAUDAKIA* AND *PARALAUDAKIA* (REPTILIA: AGAMIDAE) IN PAKISTAN**MUHAMMAD KHISROON<sup>1</sup> AND JAVEED FAROOQI<sup>1,2</sup><sup>1</sup> *Department of Zoology, Faculty of Life Sciences, University of Peshawar, Peshawar,*<sup>2</sup> *Department of Zoology, Faculty of Science, Shaheed Benazir Bhutto University, Dir,*

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The systematics of the two genera of lizards; *Laudakia* and *Paralaudakia*, in Pakistan has been revised and distribution ranges of the species of the genera have been identified. In the study the specimens collected from the various regions across Pakistan and the preserved specimens place in the museums have been studied for eight morphometric, nine meristic and five qualitative characteristics. The characteristics has been analyzed statistically in SPSS and PAST softwares and, Descriptives, ANOVA, Principal Component Analysis and Discriminants Function Analysis have been applied. The statistics has been applied for intraspecific variations and interspecific variations. Moreover, the distribution of the genera *Laudakia* and *Paralaudakia* has been revived and maps have been prepared showing the distribution of the aforementioned genera in Pakistan. The GIS software, Arc GIS, has been used to draw the maps of the species distribution. The distribution maps are representing the altitudinal, longitudinal and latitudinal distribution of the collected specimens. The study revealed that six species of *Laudakia*; *L. agrorensis*, *L. melanura*, *L. nupta*, *L. pakistanica*, *L. tuberculata* and *L. nuristanica*, and three species of *Paralaudakia*; *P. microlepis*, *P. caucasia* and *P. himalayana* are found in Pakistan. The *L. lirata* and *P. badakhshana* are poorly recorded from Pakistan and *L. nuristanica* is penetrating the northern districts of Khyber Pakhtunkhwa from Afghanistan. The study find out that the intraspecific variations and interspecific variations in the representative species of *Laudakia* and *Paralaudakia* in Pakistan are distinctive and vary from the rest of the World. The range extension of the many species and subspecies are reported and it is concluded that the many species are found in inclusive manner, e.g. the *Paralaudakia caucasia* and *Paralaudakia himalayana* are crossing the previously described boundaries

**MISGURNUS ANGUILLICAUDATUS (CANTOR, 1842); FIRST REPORT FROM PAKISTAN**

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The present study was conducted for the identification of an anonymous fish species found in Azakhel Botanical Garden (Nowshera) buried under the soil. A total of 77 specimens were collected. A batch of nine specimens was taken randomly and 30 characters along with meristic counts were taken for analysis and studied against four different norms. In standard I (Shimizu and Takagi, 2010), 15 parameters were considered and their percentages were taken against: Standard length, Head length, caudal peduncle length while ratios were also found in some of the parameters. The result of this standard was 100% concurrent with standard I. For standard II (Fowler, 1924 and Nichols, 1925), nine parameters were scrutinized and the result was falling 77.77% in the expected array while deviation from the standard was only 22.23%. In addition to this the morphological appearance, presence of asterisks, microscopic dusky spots on body, low adipose crest, lateral upper bands, presence of lamina circularis, and some other parameters referred the new reported species as diploid *Misgurnus anguillicaudatu*

**COMPARISON OF SNAIL MEAL VERSUS FISH MEAL BASED DIETS ON THE GROWTH AND BODY COMPOSITION OF CATFISH (*PANGASIUS HYPOTHALAMUS*)**

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The study was conducted to evaluate the growth performance and body composition of Catfish (*Pangasiushypophthalmus*) fry, fed with fish meal and snail meal based diet in fiberglass aquariums for three months. Experimental fish (n=25) was stocked in fiberglass aquaria (Dimensions: L, 1ft 18 inch× W, 2 ft). The fish was fed with experimental diets (fish meal and snail meal) containing crude protein (32.85 and 32.5, respectively) at 4% of fish wet body weight. After 3 months study period, the increase in weight gain due to the consumption of snail meal and fish meal was recorded as 5.57g and 5.89 g, respectively. The diet with fish meal has showed statistically significant higher growth than that of the snail meal. The feed conversion ratio (FCR) was statistically highly significant for fish fed on fish meal (1.99) than snail meal fed fish (2.09). Proximate analysis showed significant differences in fat content of fish fed with fish meal as compared to snail meal. No significant difference in moisture, protein, ash, fiber and phosphorus contents were found in fish meal as well as snail meal fed fish. In conclusion, the catfish fry showed best growth performance with fish meal due to its best nutrient composition and acceptability. The findings of present study suggest snail meal as a good alternative of expensive fish meal feed for catfish culture.

## SECTION - VI

### POSTER SESSION

#### INCIDENCE OF *MENACANTHUS EURYSTERNUS* (BURMIESTER, 1838) (PHTHIRAPTERA: AMBLYCERA: MENOPONIDAE) FROM PAKISTAN

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Chewing lice (Phthiraptera: Insecta) are normally host specific, but few of them parasitizing birds, are found to have more than one host species, belonging to same order or family or genus. *Menacanthus eurysternus* (Burmeister, 1838) is found on different species of two avian orders i.e. Passeriformes and Piciformes, in the world. In Pakistan, *M. eurysternus* have been recorded from different species of five families of Passeriformes only, while no Piciform bird has yet been recorded for this species from the region. It is recorded from *Corvus splendens*-House Crow (Corvidae), *Acridotheres ginginianus*-Bank Mynah (Sturnidae), *A. tristis*-Common Mynah (Sturnidae), *Sturnus roseus*-Rosy Starling (Sturnidae), *S. vulgaris*-Common Starling (Sturnidae), *Pycnonotus leucogenys*-White-cheeked Bulbul (Pycnonotidae), *Pycnonotus cafer*-Red-vented Bulbul (Pycnonotidae), *Passer pyrrhonotus*-Sindh Sparrow (Passeridae) and *P. domesticus*-House Sparrow (Passeridae) from different localities of Pakistan, mainly Punjab and Sindh region.

#### BIODIVERSITY OF FAMILY PIERIDAE (RHOPALOCERA: LEPIDOPTERA) OF HYDERABAD REGION

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The butterflies of family Pieridae commonly called Pierid butterflies. They are medium sized and mostly white, yellow or orange in coloration. The adults of the Pieridae are very good pollinators but the larvae or caterpillars of the some species are serious pests of the crops, vegetables and fruits. A total of 823 specimens were collected with the help of the insect net from different locations of the Hyderabad region during January August 2014 to July 2015. The identification was done with the help of the relevant literature. During the study period, ten species namely *Colotis amata* (Fabricius), *Colotis danae danae* (Fabricius), *Colotis etrida* (Butler), *Colotis protractus* (Butler), *Colotis vestalis* (Butler), *Catopsila florella* (Fabricius), *Catopsila pomana* (Fabricius), *Catopsila pyranthe pyranthe* (Linnaeus), *Anapheis aurota* (Linnaeus) and *Eurema hecabe simulata* (Moore) of two subfamilies (Pierinae and Coliadinae) were identified. The genus *Colotis* were recorded highest number (52%) including five species followed by *Catopsila* (36%) including three species and Genus *Eurema* and *Anapheis* (6%) including one species respectively. This is the first record of Pierid butterflies from Hyderabad region, Sindh, Pakistan.



### SPORADIC GOITRE PREVALENCE AND ITS ASSOCIATION WITH IODINE DEFICIENCY IN DRINKING WATER OF PLAIN AREAS OF HYDERABAD, PAKISTAN

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Thyroid gland is located in the human neck and the enlargement of this gland is known as goitre. When the supply of iodine to the thyroid gland is inadequate for the formation of sufficient quantities of thyroid hormones, goitre appears. In the present study prevalence and epidemiological risk factors of goitre in Hyderabad city and adjoining areas has been studied. The important findings of this study are summarized as follows: Females were seen to be more prone to develop goitre than males, with an overall female to male ratio of 5:1. In both sexes, the prevalence of goitre was the maximum in 15-25 year age group. Family history was positive in 22.45% cases. Of the goitre patients 77.22% belonged to Hyderabad city and 84.85% were sedentary by occupation. Goitre was more prevalent in surface water drinkers (80.75%) than in ground water consumers (19.25%), in the consumers of mine salt (67.55%) than that of sea salt (32.45%). The screening of goitre patients by blood chemistry revealed that 5.17% and 2.50% cases had hyperthyroid and hypothyroid respectively. Nodular non-toxic goitre was found in 6.71% cases, nodular toxic in 0.28% cases and solid adenoma/cyst in 24.45% cases. It seemed essential to monitor iodine content in water of this area to ascertain whether the iodine deficiency is the cause of sporadic goitre. As iodine content in water is generally taken as an index of the iodine intake, therefore 100 water samples were collected and analyzed by ion selective electrode method. Results showed that iodine contents in water are within permissible limit i.e. not lower than 3µg/L. This indicates that iodine deficiency in water of study area is not an isolated epidemiological phenomenon for goitre genesis.

### EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF AQUEOUS, ETHANOLIC AND METHANOLIC EXTRACTS OF *NIGELLA SATIVA* (KALONJI) AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIAL ISOLATES

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*Nigella sativa* (kalonji) has been used since ancient times as a nutritional supplement and for the treatment of various infections and chronic ailments. As the pathogens are becoming resistant to most of the drugs, kalonji can be used as an alternative compound to be used in modern medicines. The antimicrobial activity of kalonji was determined through disc diffusion and agar well diffusion method. All types of extracts of kalonji showed antimicrobial activity against all bacterial isolates with different zones of inhibition. In case of disc diffusion method, methanolic extract showed maximum antimicrobial activity against *B. subtilis*, *B. cereus* and *Corynebacterium sp.* Ethanolic extract showed highest antimicrobial activity against *E. coli*. Both ethanolic and methanolic extracts represented maximum antimicrobial activity for *Enterococcus sp.* In case of agar well diffusion method, ethanolic extract showed maximum antimicrobial activity against all of the bacteria except *E. coli*, where methanolic extract exhibited the maximum activity. Aqueous extract gave the least activity against all bacterial strains through both methods except for *S. aureus* by disc

diffusion method where it exhibited the maximum antimicrobial activity. The statistical analysis showed the agar well diffusion method ( $M=17.46 \pm 2.02$  mm) and methanolic extract ( $M=16.08 \pm 3.61$  mm) to be most effective against bacteria. The aqueous extract ( $M=12.75 \pm 2.90$  mm) showed least activity. The *Enterococcus sp.* ( $M=16.75 \pm 3.50$  mm) was found to be most sensitive and *S. aureus* ( $M=12.75 \pm 6.04$  mm) was found to be least sensitive to the extracts of kalonji. Phytochemical screening showed the presence of flavonoids, tannins, saponins, alkaloids and steroids depicting the antimicrobial and antioxidant properties of kalonji.

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#### MOLECULAR GENETICS OF BETA THALASSEMIA IN PUNJAB

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Beta thalassemia is found to be most prevalent disease throughout the world. This phenotype is genetically due to the mutations in the beta globin gene in the hemoglobin molecule. IVS-1 mutation is a very common mutation and is associated with 90% of the phenotype appearance. 48 samples from the Punjab were taken. DNA was isolated from the blood. The DNA was amplified by using ARMS primers one normal primer and one mutant primer. The mutational analysis of the beta globin gene was done by using amplification refractory mutation system (ARMS) technique associated with the polymerase chain reaction (PCR). Many of the patients were having the mutation and some were homozygous for the normal allele. The screening of the mutation was effective in the determination of the heterogeneity in the Punjab. This screening could be very useful for the detection and the information of the family risks associated with the beta thalassemia disease.

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#### IMPACT OF LOOP RESIDUES OF THE RECEPTOR BINDING DOMAIN OF *BACILLUS THURINGIENSIS* CRY2Ac11 TOXIN ON INSECTICIDAL ACTIVITY

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*Bacillus thuringiensis* is an excellent candidate to be used as bio-pesticide. Various techniques have been used to exploit *Bt* genes for our own benefits. In the present work, detailed study on the receptor binding epitopes of *Bacillus thuringiensis* Cry2Ac11 toxin is done by inducing different mutations in loop 2 of Domain II. The mutants are characterized to estimate the effect of mutations on the toxicity and insecticidal activities by performing bioassays against Pakistani populations of *Culex quinquefasciatus*, *Aedes aegypti*, *Helicoverpa armigera* and *Spodoptera litura*. The results revealed that Cry2Ac11 is not toxic to local populations of *Aedes aegypti* but two of its mutants with G384D and A386N (30% mortality) T387D (6% mortality) showed some toxicity. Against *Culex quinquefasciatus* and *Helicoverpa armigera*, Cry2Ac11 showed 46% and 8.35% mortality respectively with all mutants showing variable percentage mortalities. Cry2Ac11 and its mutants showed only growth retardation in local populations of *Spodoptera litura*.

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**A NEW SPECIES OF ACANTHOCEPHALA IN A CUCULID BIRD FROM SINDH,  
PAKISTAN**

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A new acanthocephalan *Centrorhynchus pakistaniensis* collected from a Cuculid bird is proposed on the basis of body shape and size; number of longitudinal rows of proboscis hooks, number of hooks in each row of proboscis; length ratio of proboscis receptacle and lemnisci; shape, position and arrangement of testes; distance between testes and proboscis, testes and posterior extremity and egg size. On the basis of these differentiating characteristics a new species *Centrorhynchus pakistanensis* is proposed.

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