

PROCEEDINGS
OF
PAKISTAN CONGRESS OF ZOOLOGY

Volume 37, 2017

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



THIRTY SEVENTH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

DEPARTMENT OF ZOOLOGY, GC UNIVERSITY, FAISALABAD

FEBRUARY 28 – MARCH 2, 2017

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PROCEEDINGS OF THE CONGRESS

Editor

Dr. A.R. Shakoori

Composed & Designed by: Fakhar Mahmood Shahid

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ACKNOWLEDGMENTS

Government College University, Faisalabad hosted the 37th Pakistan Congress of Zoology (International).

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

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37th PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

GC UNIVERSITY, FAISALABAD

FEBRUARY 28, 2017 – March 2, 2017

PROGRAMME

TUESDAY, FEBRUARY 28, 2017

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, GC University, Faisalabad
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: Prof. Dr. Iftikhar H. Qureshi

- Speakers: 1. **Ram C. Bhujel**
Aqua-Centre, Asian Institute of Technology (AIT), Thailand.
Conservation and Wise Use of Aquatic and Terrestrial Animals
2. **Prof. Dr. Qazi Javed Iqbal**
Chairman, Department of Zoology, University of the Punjab, Lahore, Pakistan
Leading Role of Biologists for the Development of Biofuel Sector in Pakistan

3. **Hasan Huseyin Atar**
*Fisheries and Aquaculture Department, Faculty of
 Agriculture, Ankara University, Ankara, Turkey*
Turkish Fisheries and Aquaculture

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

HALL – 1

SECTION I: CELL AND MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

SESSION 1

Chairperson: Dr. Farah Rauf Shakoori
 Co-Chairperson: Dr. Abdul Rehman
 02:15 PM Paper reading
 04:30 PM Tea Break and Prayer Break (Asar)

SESSION 2

Chairperson: Prof. Dr. Syed Shahid Ali
 Co-Chairperson: Dr. Saba Irshad
 05:00 PM Paper reading
 06:25 PM Prayer Break (Maghrib)

SESSION 3

Chairperson: Dr. M. Afzal Ghauri
 Co-Chairperson: Dr. Bushra Muneer
 06:40 PM Paper reading
 08:30 PM Dinner

HALL – 2

SECTION II: PEST AND PEST CONTROL

SESSION 1

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

SESSION 2

Chairperson: Prof. Dr. Shamshad Akbar
Co-chairperson: Dr. Shebab Nasir
05:00 PM Paper reading
06:25 PM Prayer Break (Maghrib)

SESSION 3

Chairperson: Mr. Abdul Aziz Khan
Co-Chairperson: Dr. Azhar Rafique
06:40 PM Paper reading
08:30 PM Dinner

HALL – 3

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

SESSION 1

Chairperson: Dr. Abdul Aleem Ch.
Co-Chairperson: Dr. Arshid Javed
02:15 PM Paper reading
04:30 PM Tea Break and Prayer Break (Asar)

SESSION 2

Chairperson: Prof. Dr. Farhat Jabeen
 Co-Chairperson: Dr. Zafar Iqbal
 05:00 PM Paper reading
 06:25 PM Prayer Break (Maghrib)

SESSION 3

Chairperson: Prof. Dr. Naeem Tariq Narejo
 Co-Chairperson: Dr. M. Samee Mubarik
 06:40 PM Paper reading
 08:30 PM Dinner

DAY TWO: WEDNESDAY, MARCH 1, 2017

JOINT SESSION II: (Plenary Lectures)

9:00 AM

Chairman: Prof. Dr. Muhammad Ali
Co-Chairman: Prof. Dr. Javed Iqbal Qazi

- Speakers: 1. **Abdul Rauf Janjua**
Department of Zoology, University of AJ&K Muzaffarabad
**Defeating Deadly Diseases by Sensitizing Communities:
 The Role Universities Can Play in Saving Lives of Fellow
 Country Men**
2. **Abdul Shakoor Chaudhry**
*School of Agriculture, Food and Rural Development,
 Newcastle University, UK. NE1 7RU*
Alternatives to Antibiotic as Feed Additives
3. **Dr. Zahid Beg Mirza**
H. # 229-B, St. # 4, F-10/3, Islamabad 44000
**Study of Some Anthropogenic Negative Impacts on the
 Wetland Ecology of Birds in the Punjab Province of
 Pakistan**

HALL – 1

SECTION I: CELL AND MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

SESSION 4

Chairperson: Prof. Dr. Tayyaba Sultana
Co-Chairperson: Dr. Soumble Zulfiqar
10:30 AM Paper reading
11:45 PM Tea Break

SESSION 5

Chairperson: Prof. Dr. Qazi Javed Iqbal
Co-Chairperson: Dr. Dil Ara Bukhari
12:00 PM Paper reading
01:30 PM Lunch and Prayer (Zuhar)

SESSION 6

Chairperson: Dr. Shahid Nadeem
Co-Chairperson: Dr. Laiq Ahmad
02:00 PM Paper reading
04:30 PM Tea Break and Prayer (Asar)

SESSION 7

Chairperson: Prof. Dr. Sarwat Jahan
Co-Chairperson: Dr. Bibi Nazia Murtaza
05:00 PM Paper reading
08:00 PM General Body Meeting
08:30 PM Dinner / Cultural Night

HALL – 2**SECTION III: ENTOMOLOGY****SESSION 1**

Chairperson: Prof. Dr. Nusrat Jahan
Co-Chairperson: Dr. Bilal Rasool
10:30 AM Paper reading
11:45 PM Tea Break

SESSION 2

Chairperson: Prof. Dr. Riffat Sultana
Co-Chairperson: Dr. Samina Qamar
12:00 PM Paper reading
01:30 PM Lunch Break and Prayer Break (Zuhar)

SECTION IV: PARASITOLOGY**SESSION 1**

Chairperson: Dr. Asmatullah Kakar
Co-Chairperson: Prof. Dr. Aneela Zameer Durrani
02:00 PM Paper reading
04:30 PM Tea Break and Prayer Break (Asar)

SESSION 2

Chairperson: Prof. Dr. Syed Akram Shah
Co-Chairperson: Dr. Nasreen Muzaffer
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 4

Chairperson: Prof. Dr. Afsar Mian
Co-Chairperson: Dr. Shabana Parvez
10:30 AM Paper reading
11:45 PM Tea Break

SESSION 5

Chairperson: Prof. Dr. M. Siddiqui Awan
Co-Chairperson: Dr. Salma Sultana
12:00 PM Paper reading
01:30 PM Lunch Break and Prayer Break (Zuhar)

SESSION 6

Chairperson: Prof. Dr. Muhammad Akhtar
Co-Chairperson: Dr. Khizar Sami-Ullah
02:00 PM Paper reading
04:30 PM Tea Break and Prayer Break (Asar)

SESSION 7

Chairperson: Prof. Dr. Naureen Rana
Co-Chairperson: Dr. Abdul Majid Khan
05:00 PM Paper reading
08:00 PM General body meeting
08:30 PM Dinner / Cultural Night

DAY THREE: THURSDAY, MARCH 2, 2017**HALL – I****SECTION 1: CELL AND MOLECULAR BIOLOGY,
PHYSIOLOGY, GENETICS****SESSION 8**

	Chairperson:	Dr. Irfan Zia Qureshi
	Co-Chairperson:	Dr. Raazia Tasadduq
09:00 AM	Paper reading	
11:30 AM	Tea Break	

SESSION 9

	Chairperson:	Dr. Shahid Baig
	Co-Chairperson:	Dr. Asma Ashraf
12:00 Noon	Paper reading	
02:00 PM	Lunch Break and Prayer Break (Zuhar)	

HALL – 2**SECTION I: CELL AND MOLECULAR BIOLOGY,
PHYSIOLOGY, GENETICS****SESSION 10**

	Chairperson:	Dr. Zulfiqar Ali Saqib
	Co-Chairperson:	Dr. M. Hafeez-ur-Rahman
09:00 AM	Paper reading	
11:30 AM	Tea Break	

SESSION 11

	Chairperson:	Dr. Nadeem Shaikh
	Co-Chairperson:	Dr. Syed Makhdoom Hussain
12:00 Noon	Paper reading	
02:00 PM	Lunch Break and Prayer Break (Zuhar)	

HALL – 3

SECTION V: PARASITOLOGY

SESSION 3

Chairperson: Dr. Aly Khan
Co-Chairperson: Dr. Sanjota Nirmal Das
09:00 AM Paper reading
11:30 AM Tea Break

**SECTION I: CELL AND MOLECULAR BIOLOGY,
PHYSIOLOGY, GENETICS**

SESSION 12

Chairperson: Dr. Saiqa Andleeb
Co-Chairperson: Dr. Faiza Saleem
12:00 Noon Paper reading
02:00 PM Lunch Break and Prayer Break (Zuhar)

CONCLUDING CEREMONY

03:00 PM Recitation
03:05 PM Congress Report by President ZSP
03:15 PM Award Ceremony
03:45 PM Concluding Remarks by the Chief Guest
03:55 PM Vote of Thanks

MEMBERS OF THE CONGRESS

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Naz, F.
Naz, U.
Rahman, I.
Salman, M.

Shehzad, F.H.
Suleman
Wahab, F.

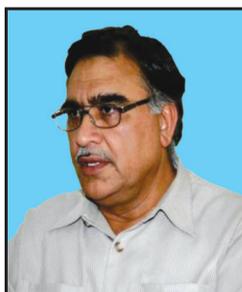
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Solangi, B.K. (Dr.)

UTHAL (Balochistan)

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CITATIONS

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2017****Prof. Dr. Syed Shahid Ali**

*Institute of Molecular Biology & Biotechnology
The University of Lahore, Lahore*

Dr. Syed Shahid Ali obtained his M.Sc. degree in Zoology in 1978 and Ph.D. degree in 1990 from the University of the Punjab. In 1988, he was appointed as lecturer in Zoology Department, University of the Punjab, Lahore, and later was selected as Professor of Zoology in 2003. His major contributions in Zoology was in the areas of Biochemistry, Toxicology of pesticides, heavy metals & various plant extracts, Bacterial enzyme production, and studies on the biochemical basis of development of resistance in stored grain pest populations. In 2006, he was appointed as a member Advanced Studies & Research Board (AS&RB) and in 2007 as Director, Research & Development (R & d), University of the Punjab Lahore. He was Editor of Punjab University Journal of Zoology which is now HEC-approved journal.

After retirement from Punjab University, Prof. Ali joined Institute of Molecular Biology & Biotechnology (IMBB), University of Lahore (UOL) as Professor, the position which he still holds. In UOL he instituted the first Research journal of the University as an Editor entitled “Pakistan Journal of Molecular Medicine”. He has also worked on many research projects as Research officer & Principal investigator sponsored by Pakistan Science

Foundation (PSF), National Scientific Research development Board (NSRDB), Higher Education commission (HEC) and University of the Punjab. He has published 110 research articles in journals of national and international repute along with many books and manuals.

Dr. Ali remained actively associated with various activities of Zoological Society of Pakistan (ZSP) as member Executive Council, Treasurer, Joint Secretary, Vice President (N) and also as member of various Organizing Committees for holding zoological congresses since eighties.

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2017**



Dr. Nasreen Muzaffar

*Incharge, Honey Bee Research Programme,
University of the Punjab, Lahore.*

Dr. Nasreen Muzaffar has done M.Sc. (Zoology), M. Phil. (Biology) and Ph.D. on honeybee management in Pakistan from University of the Punjab. She has more than 40 years of research experience and published 107 publications in national and international Journals including three books and 36 Technical Reports. She started her career from the Commonwealth Institute of Biological Control and worked on insect management of pests of crops, fruit and forest trees through biological control. The results of her research accomplishments were applied abroad for the control of some insect pests of fruit trees. Her work was also commended by the then US President Nixon in his report on the “Food for Peace Programme”. She retired as Chief Scientific Officer, Honeybee Research Programme, National Agricultural Research Centre, Islamabad and is now employed as Associate Professor on contract in the Department of Zoology, University of the Punjab.

The European bee introduced in the country under her Beekeeping Research Project has multiplied up to more than one million owing to her concerted efforts. The research work conducted on introduction of the European bee *Apis mellifera* in the country and the development of new techniques for the control of pests and diseases of honeybees was appreciated in various international competitions organized by Apimondia, an International Beekeeping Organization. She was awarded one gold medal, one silver medal and three bronze medals from top most authorities on

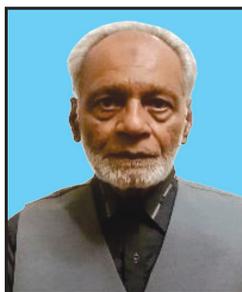
beekeeping. One gold medal was also conferred on her by Farm Guide Humdarad Foundation on the outstanding research achievements.

Dr. Muzaffar is continuing with her activities on teaching and research on honeybee breeding, control of pests and diseases using alternative methods, bee management for honey and other by-products production.

The skill trainings and workshops organized by her to disseminate this research-based technology among graduate and post-graduate students, landless farmers, other low income groups, progressive farmers and Officers of both Federal and Provincial Research and Extension Organizations has given fillip in adoption of beekeeping technology, on sound-scientific lines, by various communities.

The sidder ('ber') honey produced in Pakistan is being exported and considered to be of highest quality in the international market. Thus, the economic impact of research work done by Dr. Nasreen Muzaffar is visible in the form of several income generating programmers in operation by various Governmental and Non-Governmental Organizations.

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2017**



Abdul Ghaffar Khan

Mr. Abdul Ghaffar Khan obtained his M.Sc. Zoology with special paper in Entomology from University of the Punjab in 1964 and joined Commonwealth Institute of Biological Control, Rawalpindi as an Entomologist, where he worked on insect fauna of corn, cotton, rice, sugarcane, wheat, vegetables, fruit trees and various terrestrial and aquatic weeds during 1964 – 1992. He was then hired as Principal Scientific Officer by Pakistan Agricultural Research Council (PARC), Islamabad for development of biological control-based Integrated Pest Management for cotton pests till his retirement in 1999. During his stay in PARC, he established Biocontrol Laboratory of PARC- Regional Research Station at the University College of Agriculture, Bahuddin Zakaria University, Multan.

Mr. A.G. Khan worked under the supervision of Drs. F.J. Simmond, F.D. Bennett, D. Greathead and J. Waage Directors of Commonwealth Institute of Biological Control (CIBC) with the reputed Entomologists like Drs. M.A. Ghani, A.I. Mohyuddin, M.N. Beg, G.M. Baloch, A.A. Hashmi and G. Jilani.

Mr. A.G. Khan developed techniques and equipment for mass rearing of *Trichogramma* spp. (1979) and *Chrysoperla* spp. (1994) in Pakistan. These developments helped to uplift the biocontrol programme from experimental stage to large scale application on various crops in farmer's fields. He

imparted one month long training on mass rearing of *Trichogramma* and *Chrysopa* species to the entomologists from Bangladesh, Bhutan, Nepal, Sri Lanka and NARC, Islamabad at PARC Regional Research Station Multan. In 1999 he donated essential equipment with nucleus culture of *Trichogramma chilonis* and *Chrysoperla carnea* to National Agriculture Research Centre (NARC), Chak Shezad, Islamabad to enable them to start biocontrol programme.

After retirement Mr. Khan became consultant of Kings Group of Industries, Karachi and several Sugar Mills including Brothers Sugar Mills, Chasma Sugar Mills, Consolidated Sugar Mills, Facto Sugar Mills, Faran Sugar Mills, Haseeb Waqas Sugar Mills, Kohinoor Sugar Mills, Tandlianwala Sugar Mills, Yousaf Sugar Mills, and helped establishing Biocontrol Laboratories during 1988 -2013. In 2008, Mr. Khan was awarded Gold Medal by Pakistan Society of Sugar Technologist (PSST) for best paper on “Life history of New Species of *Chrysopa*, *Chrysoperla scelestes* Banks found associated with sugarcane insect pests in Pakistan”. Currently, he is mostly living in Louisiana, USA.

He has produced 8 Final Technical Reports, published 31 research articles and 3 books.

**RECIPIENT OF
ZOOLOGIST OF THE YEAR AWARD 2017**



Prof. Dr. Muhammad Ali (T.I.)

Vice Chancellor, Government College University, Faisalabad.

Prof. Dr. Muhammad Ali, obtained MSc (1987) with distinction from University of the Punjab, and Ph.D. in 1999 from University of Wales, Aberystwyth (UK). He spent a period of 2 years (2008-2009) in UM, Columbia, USA, for his post doctoral work in Biological Sciences (Fisheries, Molecular Biology).

Before, assuming his present position as Vice Chancellor, he spent about 28 years as a faculty member, a period which culminated into a position of Professor in 2006.

Prof. Dr. Ali has received several awards during his academic career: Merit Scholarship in MSc Zoology (distinction), Best Young Scientist in Europe Award in Italy, Star Award 2000 (South Asia Publications), Best University Teacher Award for 2001, 2005, 2011, Prof. Dr A.R. Shakoori Gold Medal (2002) by ZSP, Agha Hasan Ali Abidi Gold Medal, Prof. Dr Mirza Azhar Beg Gold Medal (2004), Farogh-e-Taleem Gold Medal Award 2011 and most prestigious National Civil Award “Tamgha-e-Imtiaz”. More than 15 research projects were awarded by different national and international agencies.

He has produced 11 Ph.Ds. and 59 M.Phil, and presently 15 Ph.D. and 7 M.Phil are registered with him. He has published 181 research articles with Total Impact Factor of 193.015. He has also published one book and two chapters of two books along with two patents.

*Other applicants of this award were Prof. Dr. Aneela Zameer Durrani, Lahore, Prof. Dr. Muhammad Ali (TI), Faisalabad, Dr. Inamullah Khan, Peshawar

**RECIPIENT OF
PROF. DR. A.R. SHAKOORI GOLD MEDAL 2017**



Dr. Muhammad Usman Rashid
*Senior Research Scientist & Head,
Basic Sciences Research, Shaukat Khanum Memorial Cancer Hospital
and Research Centre, Lahore*

Dr. Muhammad Usman Rashid studied medicine at the Punjab Medical College, Faisalabad and received his MBBS degree from the University of Punjab, Pakistan (1999). Later he registered himself as Ph.D. scholar in University of the Punjab and completed his doctoral research work in German Cancer Research Centre (DKFZ), Heidelberg, Germany (2002-2005) and obtained Ph.D. degree from University of the Punjab, Lahore in 2006. He continued to work as a Post Doc and Guest Scientist at the DKFZ, Heidelberg (2006-2013). He joined Shaukat Khanum Memorial Cancer Hospital and Research Centre as a Research Scientist (2006-2015) and established the first Cancer Genetic Lab in Pakistan. Currently he is working there as a Senior Research Scientist & Head of Basic Sciences Research (2015- to date). He also holds position of Adjunct Associate Professor in University of Health Sciences.

Dr. Rashid has received highly prestigious international awards from the Union of International Cancer Control (UICC) Geneva, Switzerland including Yamagiwa-Yoshida Memorial International Cancer (YY) award (2009);

*Other applicants of this award were Dr. Syed Akhtar Ali, USA, Dr. Marium Tariq, Karachi, Dr. Muhammad Usman Rashid, Lahore, Dr. Riffat Sultana, Jamshoro, Dr. Abdul Rehman, Lahore

UICC-International Cancer Technology Transfer award (2007); European Multidisciplinary Cancer Congress Fellowship (2011); and International Network for Cancer Treatment & Research (INCTR) Fellowship (2009). He has also received several grants from the Shaukat Khanum Memorial Cancer Hospital and Research Centre; German Cancer Research Centre (DKFZ), Heidelberg, Germany; Union of International Cancer Control (UICC) Geneva, Switzerland; Institute of Cancer Research, London, UK and Pakistan Science Foundation.

Dr. Rashid is an HEC-Approved PhD Supervisor. To date, 6 M.Phil. and 2 Ph.D. degree research projects have been completed under his supervision. He has published widely in peer-reviewed international scientific journals including JAMA, JNCI, Nature Communications and Nature Genetics and has >40 research papers to his credit.

Currently he is representing Pakistan in three international Breast Cancer Consortia. He is trying to develop a better understanding of breast, ovarian, colorectal and pancreatic cancer in Pakistani population. Only his group offers free comprehensive BRCA1/2, TP53, CHEK2, RAD51C, RAD51D, MLH1, MSH2 and MSH6 genes screening in Pakistan.

**RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2017**

4.



Dr. Arshad Javid

Assistant Professor,

*Department of Wildlife & Ecology, University of Veterinary and
Animal Sciences, Lahore.*

Dr. Javid is a wildlife biologist. He has published 23 international and 45 national research papers and 3 books. As supervisor, he has produced 6 Ph. D. students and currently supervising another 5 Ph. D. students. Currently he is working on a project 'Propagation of Ducks Rearing in Punjab' funded by the Government of Punjab amounting Rs.45.030 Million.

*Other applicants of this award were Dr. Arshad Javid, Lahore, Dr. Shaukat Ali, Muzaffarabad.

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

1. Muzaffer Ahmad Gold Medal 2017

The 24th Muzaffer Ahmad Gold Medal 2017 was received by Tayyaba Bibi a student of the University of the Punjab, Lahore for standing first in the recent M.Sc. Zoology examination.



Ms. Tayyaba Bibi

2. Afsar Mian Gold Medal 2017

Ninth Afsar Mian Gold Medal 2017 was given to Miss Hira Farhat, a student of the Arid Agriculture University, Rawalpindi for standing first in the recent M.Sc. Biology/Zoology examination.



Ms. Hira Farhat

3. Muhammad Afzal Hussain Qadri Memorial Gold Medal 2017

The 20th Muhammad Afzal Hussain Qadri Memorial Gold Medal 2017 was awarded to Faiqa Afaq a student of Karachi University for standing first in the recent M.Sc. Zoology examination



Ms. Faiqa Afaq

4. Prof. Dr. S.N.H. Naqvi Gold Medal 2017

The 13th Prof. Dr. S.N.H. Naqvi Gold Medal 2017 was given to Dr. Musarrat Memon for obtaining Ph.D. degree in Zoology with specialization in the field of Toxicology from University of Karachi.

5. M.A.H. Qadri Memorial Gold Medal 2017

The 17th Dr. M.A.H. Qadri Memorial Gold Medal 2017 was given to Dr. Erum Khadija for obtaining Ph.D. degree in Zoology with specialization in the field of Parasitology from University of Karachi.



Dr. Erum Khadija

6. Mujib Memorial Gold Medal 2017

The 24th Mujib Memorial Gold Medal 2017 was given to Saba Ishaque, student of Karachi University for standing first in the recent M.Sc. Zoology examination with specialization in Parasitology



Ms. Saba Ishaque

7. Prof. Dr. Muhammad Ali Gold Medal 2017

The 1st Prof. Dr. Muhammad Ali Gold Medal 2017 was awarded to Muhammad Usman Din a student of Government College University, Faisalabad for standing first in the recent M.Sc. Zoology examination.



Mr. Muhammad Usman Din

8. Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2017

The 1st Prof. Dr. Syed Iftikhar Hussain Jafri Gold Medal 2017 was awarded to Dilawar Ali, student of University of Sindh, Jamshoro for standing first in the recent final B.S. Examination of Freshwater Biology & Fisheries.



Mr. Dilawar Ali

9. Ahmed Mohiuddin Memorial Gold Medal 2017

The 12th Ahmed Mohiuddin Memorial Gold Medal 2017 was awarded to Miss. Iqra, student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination.



Ms. Iqra

10. Prof. Dr. S.S. Akbar Memorial Gold Medal 2017

The 4th Prof. Dr. S.S. Akbar Memorial Gold Medal 2017 was awarded to Erum Javed a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination with specialization in Entomology.



Ms. Erum Javed

Some Glimpses of Academic Sessions and The Congress Participant



Some Glimpses of Academic Sessions and The Congress Participant



CATEGORIZATION OF COMMERCIAL CANOLA CULTIVARS RESISTANT TO APHIDS (*L. ERYSIMI* K.) THROUGH PHENOTYPIC CHARACTERS

NOOR MUHAMMAD AND SHAH ALAM KHAN

*Department of Plant Protection, Faculty of Plant Protection Sciences,
University of Agriculture, Peshawar*

Abstract.- The objective of the study was to develop insect pest management strategy by exploring host plant resistance in ten canola crop *Brassica napus* L, viz, Rainbow, Omega, KS-75, Dunkled, Shiralee, Abaseen, Hoyla-401, Oscar and Zahoor. The resistance against aphid (*L. erysimi*) experiment was conducted at New Developmental farm laid out in randomized complete block design (RCBD), The University of Agriculture Peshawar during winter season of 2015-16 and 2016-17. Mean of overall result shows that minimum aphid population (162.0/plant) were recorded on KS-75 and maximum aphid population (367.7/plant) were recorded on Abaseen cultivar. The appearance of natural enemies of aphid on germplasm were noted on last week of 1st month of the calendar both years 2015-16 and 2016-17, (50 DAS after introduction) and ended till March end or early April (130 days after sowing). Minimum, maximum and average temperature correlation coefficient (r) showed a positive effect but mean relative humidity showed significantly negative effect. KS-75 possess some levels of resistance whereas in response to plant characters including no. of sub-branches/plant, no. of pods/plant, length of pod, no. of grain/pod, plant height, thousand seed weight & plant maturation were found significantly better genotype KS-75 as compared to the other tested cultivars.

Keywords: Aphid losses, Canola germplasm, Correlation of aphid losses, Characteristics of plant.

INTRODUCTION

Canola (*Brassica napus* L.) belongs to the family Brassicaceae and is an important oilseed crop grown in temperate climates. It is major oilseed crop cultivated throughout the country due to its high adaptability in conventional farming systems (Mohammad and Khan, 1981). The seeds of canola contain 5-8% saturated fats, 2% erucic acid and 30µg/l of glucosinolates (Raymer, 2002).

The yield of canola crop is highly reduced by insect pests and their corresponding diseases. Major insect pests of canola in Southern Punjab, district Multan, Bahawalpur and Dera Ghazi Khan are cabbage caterpillar, leaf miner and mustard aphid (Saljoqi *et al.* 2006; Aslam and Razaq, 2007). The mustard

aphid, is undoubtedly the most destructive insect pest in canola crop (Bakhetia *et al.*, 1989) which in cluster on stems, leaves, flowers, stalks and pods. The effected leaves turn necrotic appearance and acquire a curly in leaves, and the inflorescences fails to form silique.

Several control strategies have been evolved so far to manage brassica aphids. The uses of insecticides again and again have led to the development of resistance in many pests (Sun *et al.*, 2000). The development of resistance to insecticides in pests has forced the protectionists to opt for alternate strategies. However, the most durable pest control is through integrated pest management strategy with no or little adverse effect on environment, economy, natural enemies and health hazard. Consequently, biological control is getting more attention of farmers and pest managers, which is one of the safest tools for suppressing the pest (Maurya, 1998; Kumar and Sharma, 1999).

An insect resistant cultivar (Stoner and Shelton, 1988), they have a great job to provide a knowledge of sources of resistance. Different coccinellids, syrphid fly and green lace wing are the abilities natural enemies of the *L. ervsimi* (Mathur, 1983; Kalra, 1988). Ladybird beetle larva stages feeding almost 300-400 mustard aphids to complete its lifecycle (Dixon, 2000). These type of control strategies resistance great quantify stand with injury for a given set of aphid abundance stress and prevailing environmental conditions. Further, potential to tolerate a specials level of aphid losses would also be allocated by physical structure of the preference plant as well as the level of autozygosity inherent in usually crosspollinated rapeseed and predominantly one another pollinated vegetarine forms of Brassicaceae families.

Keeping in view the canola cultivar and yield losses due to *L. ervsimi* it was needed to improve the present genotype in the local market for screening against these enemies. Thus, the aim of this study was to evaluate the germplasm for further studies on integrated pest management model, and to categorize different components of resistance (antixenosis, antibiosis or tolerance) in the selected canola cultivars by conventional method/phenotypic screening. These tools will open new avenues for plant breeders for cultivars with higher yields, tolerance to abiotic stress as well as improved insect and disease resistance.

METHODOLOGY

Allocation

The experiment was initiated at New Developmental Farm (NDF), The University of Agriculture Peshawar, Pakistan at 29.5° North latitude (width wise)

and 79.3° East longitude (length wise) and at an altitude (equator wise) of 243.04 m high, the mean sea level during crop season 2015-16. This area falls near sub-humid and sub-tropical region, which is hot and dry in summer while freezing cold in winter. The summer hotness rises up to 44°C ±5, while it can be as low as 2.0°C ±2 in winter. The RH around about 90±5% during rainy season which ends in the 2nd month of the year after which it goes up to 54% till the 4th month of the calendar year.

Meteorological data collection

Every day data were recorded on maximum temp (T max), minimum temp (T min). Average temp (T avg), relative humidity (RH) and rainfall (RF) were calculated from the available meteorological data. Every day data were then adjusted to 7 days (weekly) mean values except for rainfall.

Experimental design

The experiment performed was in a RCB Design with 4 replications. Ten different brassica genotype varieties viz. Rainbow, Omega, NARC, PARC, Shiralee, Abaseen, Hoyla-401, Oscar and Zahoor were obtained from G. M. Khattak New Block of (IBGE), The UOA. Pesh, Pakistan. Seeds were grown in lines (rows) by hand drill machine on 14th November 2015-16. Each plot (5x4 m²) of 4 lines (rows) with plant-plant of 30-40 cm and row to row distance with 75 cm, respectively. One bag of DAP use and urea accordance to use per acre in the time of sowing to the sandy loam soil.

Sampling

To manage the category of resistance, aphid abundance was recorded at 7 days intervals from the start of aphid attack till to the end of maturity. By using the sweeping net and the direct count methods, samples of 5 double strokes and 5 canola leaves were picked up weekly 50 days after plantation till harvesting at random from each experimental plot. Samples were taken in polyethylene bags to laboratory and examined by stereomicroscope. All preserved specimens were identified at Entomology Department, The University of Agriculture Peshawar, Pakistan. Half fortnight (week basis) data were calculated on the basis of counting number of aphids on top (upper), between (middle) and lower (bottom) leaves of 3 plants. When plant reached to the 4 to 6 leaves stage data was recorded. Next data was recorded when the inflorescence appeared, by counting

aphids on silique upper portion (2.8 cm). The biological control agent (parasites, parasitoids and predators) of aphids were also recorded.

Study attributes of cultivars

Data of cultivars was collected as number of branches/plant, number of silique/plant, length of silique, number of seeds/silique, thousand seeds weight, height of plants and crop maturation.

Statistical analysis

The statistical data were analysed by using ANOVA to evaluate the infestation of canola varieties on aphid population. Means were separated using LSD tested at 0.05% level of significant using statistix 8.1 package (Steel and Torrie, 1980).

RESULTS

Currently, mustard aphid (*L. erysimi*) and potato aphid (*M. persicae*) infested the plants. Colonies of aphids were found on the underside of leaves. The aphid mouthparts are of piercing and type, hence suck the plant juices and when substantial damage has occurred, the plants show necrotic appearance compared to healthy plants and their abundance is frequently in the form of spotted patches. Before plant symptoms become apparent, the damaged plants become stunted with poor canopy development and poor yield. Aphids feed continuously during reproductive stages, which results in poor silique and seed formation. When the germplasm material comprising of 10 varieties were exposed to pest during the growth period, none of plants showed complete tolerance for all the test traits. Similarly, grain yield was low and the damage was more under field condition after aphid exposure.

Mustard aphid (*L. erysimi*) and potato aphid (*M. persicae*) were calculated as primary insect attackers of canola crop. The abundance of *L. erysimi* was greater than that of *M. persicae* during two seasons study. First winged apterous appeared round about on 1st January, 2015-16 but early stage (nymphs) of both the pests were observed on January of 10th along burrows of the field. After 7 days of interval 25 mustard aphids nymphs were observed on single species of plant. First time aphids were noticed in second week of January during both seasons. However, the abundance of aphids were enough to be calculated by February 15th. Mean seven nymphs of *L. erysimi* were noted in the experiment

trial on 20th February, 2015-16 and 2016-17 in field condition on 10 varieties of *B. napus* and *B. juncea*.

Meteorological data

Table I shows minimum, maximum and average temperature as well as relative humidity during the period of initial level, peak level and decline level of aphid population. First aphids infestation was noted on January 16-01-2015-16 (60 DAS) at minimum, maximum and average temperature of 12.71, 25.14 and 18.93°C and mean RH of 91.28% (Table I). The mean aphid abundance was 0.475 aphids/10 cm in inflorescence on different varieties of canola cultivars on 102 DAS on 27-02-2015-16 the mean aphid population increased rapidly (336.13 aphids/10 cm) inflorescence on different varieties of canola cultivars, with the increase of 2.41°C average temp and relation humidity of 12.83% on 60 day after sowing. On 27th of February the peak was observed as 336.13 aphids/10 cm inflorescence in lower, higher and moderate temperature at 16.89, 27.67 and 21.34°C respectively and relative humidity of 78.45%. The graph started declining gradually towards bottom line. So, the abundance of aphid pest decreased as 60.97 aphids/10 cm inflorescence in 130 DAS with lowest, highest and mean temperature of 22.34, 30.60 and 25.84°C and mean relative humidity of 58.12%.

TABLE 1.- METEOROLOGICAL DATA DURING THE INTERVAL OF SEVEN DAYS OF INFESTATION AT NEW DEVELOPMENTAL FARM OF IBGE.

Observations date	Crop age (DAS)	Mean Temp (°C)		Average	Mean RH (%)
		Min	Max		
16.01.16	60	12.71	25.14	18.93	91.28
23.01.16	67	13.57	25.86	19.72	88.86
30.01.16	74	14.86	25.05	19.94	86.28
06.02.16	81	14.86	26.43	20.64	85.31
13.02.16	88	15.28	26.58	20.93	83.60
20.02.16	95	16.03	27.32	21.02	81.04
27.02.16	102	16.89	27.67	21.34	78.45
05. 03.16	109	18.67	28.20	22.96	72.08
12.03.16	116	18.98	28.90	23.02	70.70
19.03.16	123	20.01	29.86	24.59	64.32
26.03.16	130	22.34	30.60	25.84	58.12
02.04.16	137	23.40	32.04	26.87	54.18
09.04.16	144	24.68	34.66	28.34	49.50
16.04.16	151	25.46	35.98	29.56	44.64
Mean	105.5	18.41	28.87	23.12	68.15

*DAS-Days after sowing

TABLE II.- MEAN APHID ABUNDANCE ON DIFFERENT VARIETIES OF CANOLA CULTIVARS AT NEW DEVELOPMENTAL FARM 2015-16.

Date of observ- ation	Geno- types	Rain- bow	Omega	NARC	PARC	Shiralee	Abaseen	Hoyla- 401	Raya Anmol	Oscar	Zahoor	LSD values
16.01.16	60	0.00c	0.00c	0.00c	0.00c	1.75c	1.25c	0.25c	0.25c	0.25c	1.00c	0.52
23.01.16	67	6.75ab	6.50b	7.25ab	7.75ab	8.25a	7.00ab	6.75ab	7.00ab	7.25ab	7.50ab	1.53
30.01.16	74	17.75bc	17.75bc	16.50c	18.75ab	18.50ab	19.00ab	19.75a	18.00ab	18.25bc	18.00ab	1.52
06.02.16	81	31.25bcd	32.50ab	28.75c	32.25abc	33.25a	33.00a	32.00abc	31.00cd	30.25d	32.25abc	1.49
13.02.16	88	61.25ab	60.25b	47.75c	62.00ab	65.00ab	61.25ab	60.50b	62.50ab	64.25a	61.25ab	3.25
20.02.16	95	104.7a	104.7a	80.50b	107.5a	110.5a	110.0a	62.50a	107.2a	111.7a	110.0a	9.22
27.02.16	102	345.0bc	343.5c	162.0d	364.2ab	355.0ab	367.7a	361.2abc	357.0abc	358.5abc	346.2bc	19.61
05.03.16	109	278.9ab	276.4abc	125.7d	266.2c	274.4ab	273.9abc	269.9bc	284.2a	275.4abc	271.1bc	11.32
12.03.16	116	209.6a	204.6a	100.6b	203.9a	204.9a	203.9a	203.6a	206.4a	202.6a	207.4a	4.51
19.03.16	123	112.6abc	106.8c	79.6d	109.6bc	114.6abc	112.6abc	116.3ab	112.3abc	119.6a	108.5bc	2.43
26.03.16	130	63.16abc	59.41d	48.41c	62.66abc	63.66ab	61.91bcd	62.16abcd	63.41bc	64.91a	60.34cd	1.79
02.04.16	137	17.99bc	17.99bc	16.74c	18.99ab	18.74ab	19.24ab	19.99a	18.24bc	18.49ab	18.01bc	1.64
09.04.16	144	3.229a	3.229a	2.729a	3.729a	4.229a	2.729a	3.479a	3.479a	2.229a	4.081a	1.02
16.04.16	151	-0.001c	-0.001c	-0.001c	-0.001c	1.748a	1.248ab	0.248c	0.248c	0.248c	1.007b	0.20
Mean	10.5.5	89.44	88.11	51.18	51.99	90.89	76.48	87.04	90.78	90.97	89.04	

Means followed by different letter (s) are not significantly not different from each other at (p<0.005)

Screening of varieties

The data was calculated at the New Experimental Farm, The University of Agriculture Peshawar, Pakistan revealed that no, one of canola variety was resistant/tolerant from infest of aphids attack. Aphid abundance were started from January 3rd week 2015-16 and initiated with growth of plants and infestation caused by aphid on different canola varieties. However, the rate growth of its population dynamic was much faster with a passage of time along with a increase of temperature and slightly decrease of relative humidity during warm months (Feb to till end of Apr, 2015-16) as shown in Table II. The means of overall result of aphid density/leaf on different canola genotypes indicated that maximum abundance of (367.7) aphids/leaf was observed on variety Abaseen followed by PARC (364.2), Hoyla-401 (361.2), Oscar (358.5), Raya Anmol (357.0), Shirlee (355.0), Zahoor (346.2), Rainbow (346.0), Omega (343.2) and NARC (162.0). All tested genotypes were found non-significantly different from each other in their resistance/tolerance except NARC to developed aphid population growth. The NARC was found with the highest significant population (LSD=162.0 at $p=0.05$) of mustard aphids only on 27 Feb followed by Omega and Zahoor varieties.

Biocontrol agents of aphid

The following biocontrol agents of aphid were observed during present study:

Parasites: *Aphidius* sp. (Hymenoptera)
Parasitoids: *Diaretiella rapae*. (Hymenoptera)
Predators: Ladybird beetle (Coccinellidae; Coleoptera)
Chrysoperla sp lion aphid (Chrysopidae: Neuroptera)
Syrphid fly (Syrphidae, Diptera)

Physiological characteristics of canola cultivars

The physiological characteristics of canola cultivars tested for their resistance/tolerance against aphid infestation are given in Table III. The data revealed that the most of the varieties recorded in NARC genotypes were found significantly different from each other with respect to number of sub-branches/plant. One NARC genotype (31.2) produced sub-branches/plant and was found on the top, followed by Omega, KS-75, Dunkled, Shirlee, Abaseen,

TABLE III.- PHYSIOLOGICAL CHARACTERISTICS OF DIFFERENT CANOLA CULTIVARS TESTED FOR THEIR RESISTANCE/ TOLERANCE AGAINST APHIDS ATTACK AT NEW DEVELOPMENTAL FARM IN 2015-16.

Varieties	No. of bran/ plant	No. of pod/ plant	Length of pod (cm)	No. of grains/ pod	Plant height (cm)	Weight of 1000 seeds (g)	Plant matur- ation (days)
Rainbow	31.26a	4072.6a	7.09a	26.92a	132.3a	2.91a	145.0e
Omega	29.50a	3591.2b	6.57b	24.25a	124.7b	2.85a	145.2e
KS-75	26.75b	3228.6c	6.15c	19.29b	113.0c	2.26b	153.2d
Dunkled	25.75b	3056.5d	6.15c	19.00b	111.7cd	1.69c	155.0cd
Shiralee	25.63b	2852.2e	5.99cd	17.67bc	110.7d	1.55c	155.0bcd
Abaseen	23.38c	2836.2e	5.91cde	15.50cd	110.6d	1.45c	156.6abc
Hoyla-401	22.76cd	2769.4f	5.81de	15.25cd	109.7df	1.39cd	158.3ab
Raya Anmol	22.50cd	2763.2f	5.67ef	14.50d	109.7de	1.21cd	158.8a
Oscar	21.00de	2757.7f	5.50f	13.75d	108.3e	1.13d	158.8a
Zahoor	19.25e	2594.7g	5.46f	13.04d	108.3f	1.02d	159.9a

Means followed by different letter (s) are not significantly not different from each other ($P < 0.05$)

Hoyla-401, Raya Anmol, Oscar and Zahoor with production of 29.5, 26.7, 25.7, 25.6, 23.3, 22.7, 22.5, 21.0, and 19.2 sub-branches, respectively. Similarly all tested. NARC genotype gave significantly the most (4072.6) pods/plant, followed by Omega, KS-75, Dunkled, Shiralee, Abaseen, Hoyla-401, Raya Anmol, Oscar and Zahoor with production of 3591.2, 3228.6, 3056.5, 2852.2, 2836.2, 2769.4, 2763.2, 2757.7 and 2594.7 pods per plant, respectively. All varieties were tested for the measurement of pod length, NARC variety gave significantly great horizontal (7.09 cm) length of pods per plant, followed by Omega, KS-75, Dunkled, Shiralee, Abaseen, Hoyla-401, Raya Anmol, Oscar and Zahoor 6.57, 6.15, 6.15, 5.99, 5.91, 5.81, 5.67, 5.50 and 5.46 length of pods per plant, respectively. Similarly, all tested NARC variety gave significantly more (26.92) average no. of seeds/pod ranked in first followed by Omega, KS-75, Dunkled, Shiralee, Abaseen, Hoyla-401, Raya Anmol, Oscar and Zahoor and with produce of 24.25, 19.29, 19.00, 17.67, 15.50 and 15.25. 14.50, 13.75 and 13.04 seeds. The data was recorded that the tallest plant height in NARC genotype (132.3 cm), followed by Omega, KS-75, Dunkled, Shiralee, Abaseen, Hoyla-401, Raya Anmol, Oscar and Zahoor with the plant height of 124.7, 115.0, 111.7, 110.7, 110.7, 110.6, 109.7, 109.7, 108.3 and 108.3 cm, respectively. The height of the plant of all genotypes were found non-significantly different from each other

except NARC at 0.05% level of significance. As far as thousand seeds weight was concerned NARC genotype was ranked 1 (2.91g), followed by Omega with 2.85g. These two genotypes are non-significantly different from each other, KS-75 ranked second with 2.20g while the weights of 1000 seeds of Dunkledm Shiralee, Abaseen, Hoyla-401, Raya Anmol, Oscar and Zahoor are respectively, 1.69, 1.55, 1.45, 1.39, 1.21.1.13 and 1.02g. The NARC varieties Rainbow and Omega mature significantly early (145.0 days) compared to the rest of other varieties. The plants of KS-75, Dunkled, Shiralee, Abaseen, Hoyla-401, Roya Anmol, Oscar and Zahoor mature in 145.2, 153.2, 155.0, 1.0, 100.0, 158.3, 158.8, 158.8 and 159 days, respectively. The crop maturation of all varieties were found non-significantly different from each other except NARC at 0.05% level of significance.

DISCUSSION

Correlation coefficient (r) minimum temperature, maximum temperature and average temperature (Table 1) showed a positive effect and relative humidity negative effect with aphids abundance. Infestation of *L. erysimi* on different genotypes is largely governed by the average temperature and negatively by mean relative humidity. It was found that the winter seasons 2015-16 remained favorable for quick production of population of aphids. It was observed that the important period of preference and non-preference of the aphid population was from the mid of January to the end of March which was also reported by Prasad and Chakravarty (2000). It was also observed that in the starting phase, when the maximum temperatures were around 25°C, winged, migrant adults were observed on the crop. This temperature range was conducive to the aphid multiplication as also reported by Prasad and Phadke (1987). Our findings also agree with the reports of Singh and Malik (1998) which show that the increase in temperature was significantly favorable for aphid multiplication but relative humidity has shown negative response on its population dynamic. In the present findings, the aphids population decreased after 130 DAS during mid of March. It is due to the maturation of crop. Identical results were obtained by Singh and Malik (1998). It was further explained by Singh and Singh (1994) that maturation of crop has created net deficit in water content in plant tissues leading to food scarcity and alate formation in aphid colonies.

The influence of environmental factors on *L. erysimi* was studied by many workers at different places. Singh and Verma (1990) observed that lowest and highest relative humidity of preceding three days of observation were the most

important factors to increase the aphid abundance and out of these two, the minimum relative humidity played an important role in increasing the aphid multiplication. Rohilla *et al.* (1996) observed from the field studies conducted in Haryana that the pest incidence increased with an average temperature of about 13.7°C and a relative humidity 65%. It decreased with temperature above 35°C, relative humidity less than 60% and rain fall more than 10 mm per day. The present results were supported by Amer *et al.* (2009) and Khan and Begum (2005), who studied canola resistance/susceptible against aphid.

According to Faheem (2004), who conducted similar type experiments on these cultivars determine that other than Omega, PARC and Zahoor genotypes all other cultivars showed similar result significantly not different populations of *L. erysimi* during 2005. Similar results were observed when experiments were conducted in Multan (Aslam *et al.*, 2005). A genotype tested in present study was found to have an aphid infestation index of approximately 3 as described by Bakhetia and Sandhu (1977).

The NARC cultivar of mustard plants showed the highest characteristics i.e. no. of branches/plant, no. of pods/plant, length of pod, no. of grains, plant height and weight of thousands seed. Also the plant mature in days were observed the least in this cultivars. Although resistant/tolerant genotypes of *B. napus* against aphids have been reported in the past (Bakhetia, 1990), but it has been advocated that resistance is probably not sufficient to control significant losses in yield (Sehkon and Ahman, 1992).

CONCLUSION AND RECOMMENDATION

All the genotypes were infested with aphids and no one was completely free of aphid's infestation means that none of the genotype was absolutely resistant. The aphids appeared in late January or early February and after reaching to its peak at the end of February or first week of March its population was decreased. The differences in aphid population can be attributed to variations in their genetic makeup. The genotype NARC with 160.0 aphids/plant was least preferred by aphids over other genotypes. It can be of significant importance in varietals introduction programme as a source of resistance for further improvement of *Brassica* germplasm. This resistant genotype may help to minimize the possible use of insecticides and to improve future integrated pest management programme. In the 2nd and 3rd week of February close attention should be paid to the aphids of appearance on the brassica and control measures applied if necessary.

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Short Note

**PREVALENCE OF MALARIA IN LOCAL POPULATION OF
TEHSIL BARIKOT, DISTRICT SWAT**

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Abstract.- Present study was carried out in local population of Tehsil Barikot, District Swat for prevalence of malarial disease. A total of 997 individuals were screened for malarial test in the study area and divided into four category of age (1-15, 16-30, 31-45 and 46 onward), gender wise, union council (Kota, Barikot, Shamoza and Ghalegi) and month wise. Thick and thin blood smears were prepared and examined under microscope. It was concluded that 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 adults of age group 46 years and onward, which was 22.64%. Most of the positive cases were found in the month of November which were 24 out of 67 (35.82%) and less cases were noted in the month of May which were 12 out of 106 (11.32%). Most of the cases were found in U.C Kota with 60 positive (26.08%) out of 230 blood samples and low prevalence was found in U.C Ghalaigai with 42 positive (13.12%) out of 320 samples. Gender wise prevalence of malaria disease showed male 106 males (27.53%) out of 385, 98 female (16.01%) out of 612.

Key words: Malaria, *Plasmodium vivax*

Malaria is well known to anthropological organisms since long times predominantly in Africa as well as in Asia. In Pakistan *Plasmodium vivax* is very common (Kathryn *et al.*, 2005).

Current studies were conducted on different area of Barikot DHQ Hospital, District Swat. Collections of the blood samples were grouped in to 1-15 to 45-< age wise. Patient had clinical symptoms *i.e.* headache, vomiting, cough,

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fever, chills and pain. A proforma containing all the above information was filled in by the patients.

The present study was conducted on the incidence of malaria disease Barikot, District Swat. A total of 997 blood sample were collected. The results show that 204 were positive, whereas 793 were negative for malaria parasite. Four Union council i.e Shamoza, Ghalagai, Kota and Barikot were screened. The higher incident rate was found in Union council Shamoza 55 (40.45%) and lowest rate were found in Union council Ghalagai 42 (13.13%) (Table I and Fig. 1). Gender wise prevalence of malaria parasite were more in male 106 (27.54%) and lower prevalence were reported in female 98 (16.02%) (Table II, Fig.2). In age wise prevalence of malaria were found in children 111 (23.37%) category 1-15 age and less cases were found in adult 55 (15.6%) category 16-30 age (Table III). In month wise prevalence of malaria, high in November 24 (35.82%) and low in May 12 (11.32%) (Table IV).

TABLE 1.- UNION COUNCIL WISE PREVALENCE OF MALARIA IN TEHSIL BARIKOT, SWAT.

Union council	No of slides	+ive cases	%age
Kota	230	60	26.08
Barikot	311	47	15.11
Ghalaigai	320	42	13.12
Shamoza	136	55	40.44
Total =4	997	204	20.46

In this study children were more affected due to low socio-economic conditions, which may be due to different geographical climatic factors. The malaria disease was the second biological problem of the world (WHO, 2011). *Plasmodium falciparum* were found more dangerous and deadly in rural areas of Punjab and Muzaffargarh District (Sahar, 2012). In the current study, 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 of the cases were found in U.C Kota with 60 positive (26.08%) out of 230 blood samples and low prevalence in U.C Ghalaigai with 42 positive (13.12%) out of 320 samples. Gender wise prevalence of malaria disease shows male 106 (27.53%) out of 385 whereas female 98 (16.01%) out of 612. The studied area is generally considered to be free of malaria therefore high incidence is not expected. The study was limited by several factors and the major one for

the diagnosis of asymptomatic malaria was the microscopic diagnosis. For the low parasitemia more refined technique like PCR is more appropriate and the result will be certainly different from the present result.

TABLE II.- GENDER WISE PREVALENCE OF MALARIA IN TEHSIL BARIKOT, SWAT.

Gender	No. of Individuals	Positive	%age
Male	385	106	27.53
Female	612	98	16.01
Total	997	204	20.46

TABLE III.- AGE WISE PREVALENCE OF MALARIA PERCENTAGE IN TEHSIL BARIKOT, SWAT.

Category	No.of Individuals	Positive	%age
1-15	475	111	23.36
16-30	359	56	15.59
31-45	110	25	22.72
46-<	53	12	22.64
4	997	204	20.46

TABLE IV.- MONTH WISE PREVALENCE OF MALARIA PERCENTAGE IN TEHSIL BARIKOT, SWAT

Months	No. of slides	Positive	%age
May	106	12	11.2
June	162	14	8.64
July	96	16	16.66
August	149	16	3.39
September	229	69	30.13
October	188	53	28.19
November	67	24	35.82
Total	997	204	20.46

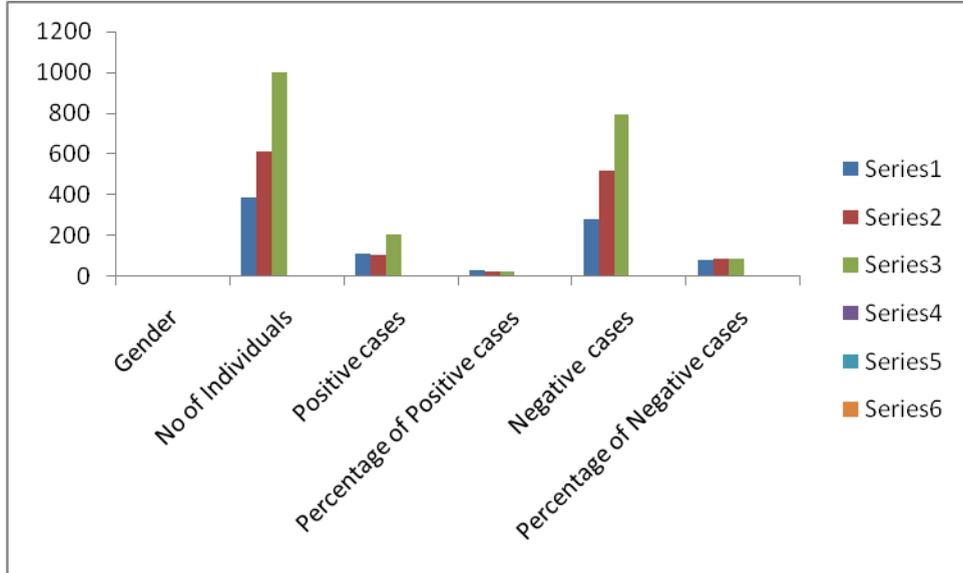


Figure 1: Gender wise prevalence of malaria percentage in Tehsil Barikot, Swat.

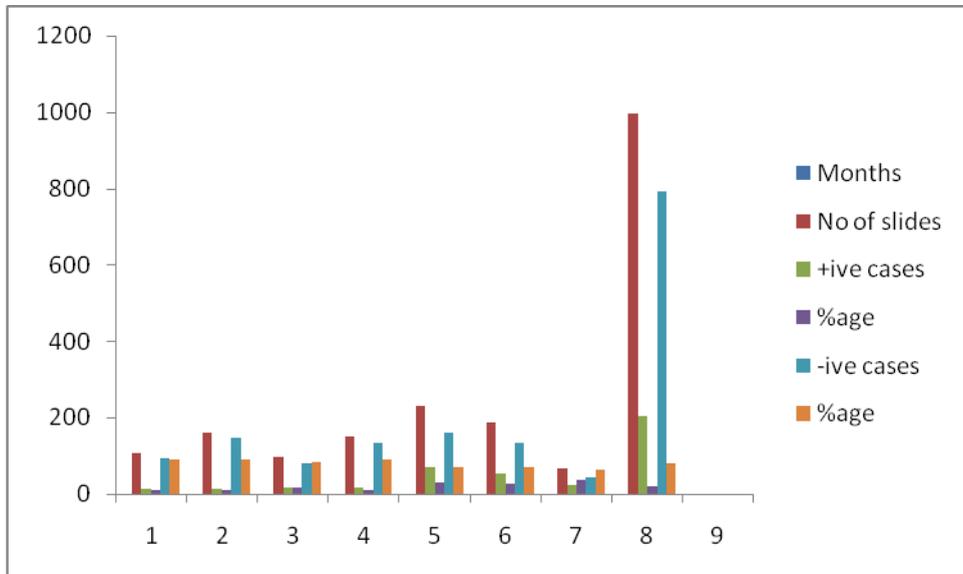


Figure 2: Month wise prevalence of malaria percentage in Tehsil Barikot, Swat.

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MANAGEMENT OF TOMATO FRUIT BORER (*HELICOVERPA ARMIGERA*) THROUGH PLANT EXTRACTS AND BIOCHEMICAL INSECTICIDES

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Abstract.- The present research was conducted at the Agriculture Research Station, Baffa (Mansehra during the normal cropping season, 2015. The experiment was laid out in randomized complete block design (RCBD) with application of seed extract of Neem (*Azadirachta indica*) with 2%, 5% and 7% concentrations, as well as Puluck (*Persicaria hydropiper*) at 1% and 3% concentrations against *Helicoverpa armigera*, tomato fruit borer. During first week, span 24h, 48h, 72h and 7 days. 7% Neem showed highest efficacy during time. Similarly, in all 8 weeks, 7% Neem followed by 5% Neem showed significantly high and moderate efficiency against *H. armigera* (0.1 and 0.18 mean number of larva) in tomato plant. Weed extract was found to be slightly effective than neem extract. It was concluded that Neem 7% being highly effective compared to locally available insecticide in the market can be recommended to tomato farmers. Further study is however, required for isolation of those compounds that enhance resistance in tomato against *H. armigera* as well as other tomato pathogens.

INTRODUCTION

Tomato (*Solanum lycopersicum*) belongs to the nightshade family, Solanaceae. It is a delicious nutritious fruit, more widely known as a vegetable. Botanically, tomato is the ovary of a flowering plant: therefore, it is a fruit or more specifically, a berry, Tomato is consumed in multiple ways, including raw, as an ingredient in many dishes, sauces, Tomato is the second most important vegetable crop next to potato in the world (Allen, 2008). The total world cultivated area was 4.8 million hectare in 2012 and production for tomato was about 161.8 million. The average world farm yield for tomato was 33.6 tons per hectare in 2012 (FAOSTAT, 2014).

The species originated in the South American Andes and its use as a food originated in Mexico, and spread throughout the world. Its many varieties are now widely grown in greenhouses in cooler climates. The plant typically grows

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to 1-3 meters (3-10 feet) in height and have a weak stem that often sprawls over the ground and vines over other plants. This herbaceous plant is perennial in tropical climate, although often grown outdoors in temperate climates as an annual (John, 2014). An average common tomato weighs approximately 100 g (4 oz) (Houchin, 2010). Tomato is now grown worldwide for its edible fruits, with thousands of cultivars, with varying fruit types and for optimum growth in differing growing conditions. Cultivated tomatoes vary in size, from tom berries, about 5 mm in diameter, through cherry tomatoes, about the same 1-2 cm (0.4-0.8 in) size as the wild tomato, up to beefsteak tomatoes 10 cm (4 in) or more in diameter. The most widely grown commercial tomatoes tend to be in the 5-6 cm (2.0-2.4 in) diameter range. Most cultivars produce red fruit, but a number of cultivars with yellow, orange, pink, purple, green, black, or white fruit are also available. Multicolored and striped fruit can also be quite striking. Tomatoes grown for canning and sauces are often elongated, 7-9 cm (3-4 in) long and 4-5 cm (1.6-2.0 in) diameter: they are known as plum tomatoes, and have a lower water content (Allen, 2008).

Tomatoes are a rich source of vitamins A and C and folic acid. Tomatoes contain a wide array of beneficial nutrients and antioxidants, including alpha-lipoic acid, lycopene, choline, folic acid, beta-carotene and lutein. One medium tomato (approximately 123g) provides 22 calories, 0g of fat, 5g of carbohydrate (including 1g of fiber and 3g of sugar) and 1 gram of protein. Lycopene is the antioxidant that gives tomatoes their rich red color. Tomatoes account for 80 percent of lycopene consumption (Megan, 2015).

Tomato crop is attacked by various insect pests *i.e.* stink bugs, cutworms, tomato hornworms and tobacco hornworms, aphids, cabbage looppers, whiteflies, tomato fruit worms, flea beetles, red spider mite, and Colorado potato beetles. Among these insect pests, tomato fruit worm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is major threat to tomato crop causing significant yield loss (Talekar *et al.*, 2006).

Tomato fruit borer (*Helicoverpa armigera*) is a major pest of tomato widely distributed in tropics, subtropics and warmer temperate regions of the world. The moth is stoutly built and is yellowish brown in colour. There is a dark area near the outer margin of each forewing. The forewings are marked with grayish wavy lines and black spots of varying size on the upper side and a kidney shaped mark and a round spot on the underside. The hind wings are whitish and lighter in colour with a broad blackish band along the outer margin. The

caterpillar cause damage and when fully grown are 3.5cm in length, being greenish with dark broken grey lines along the sides of the body. Pupa is dark brown in colour and has a sharp spine at the posterior end, while the eggs are shining greenish yellow, round and 0.46-5.3 mm in diameter (Atwal, 2004).

Eggs are laid singly, generally on leaves and flowers in the upper canopy of the plant. A female lays approximately 500 eggs, on hatching the young caterpillars feed on tender foliage, advance stage caterpillars attack the fruits. Damaging stage is larvae, feed on the foliage and late larval instars bore inside the fruits. Such fruits are not preferred by consumers. Larvae of fruit worm are polyphagous it can attack tomato fruit at any stage of growth decreasing its market value (Gajete *et al.*, 2004). In Pakistan, 32-35% fruit infestation by *H. armigera* has been reported in tomato.

The use of conventional insecticides has raised some concern about their threat to the environment and development of insecticide resistance in insects, there is an imperative need for the development of safer, alternative crop protectants such as botanical insecticides. Chemical insecticides are commonly used for the control of fruit borer showing best control of fruit borer but synthetic insecticides leave ill effect on our environment beside this, chemical insecticides for the control of this pest has led to insecticidal resistance by the pest (Nauen and Denholm, 2005). Like most other countries, Pakistan largely relies on pesticides to control pests. The use of insecticides has increased many folds since their first introduction in Pakistan's Agriculture. Indiscriminate application of pesticide during 1980s and 1990s has contributed a lot in heavy outbreaks of *H. armigera* (Ahmed *et al.*, 1997). There are now several pesticides resistant biotypes active in various cropping systems worldwide and in Pakistan. To overcome the problems of synthetic chemical hazards, one of the best control measures is the use of plant origin products. The popularity of the plant products increasing day by day because of their biodegradability, least persistence and least toxic to non-target organisms, economic and easy availability. Today about 200 plants with insecticidal activities are known (Singh *et al.*, 2001). Botanical insecticides tend to have broad spectrum activity, are relatively specific in their mode of action, and easy to process and use in farm levels. They are also safe for higher animals and the environment. Botanical insecticides can often be easily produced by farmers and small-scale industries, indigenous plant materials are cheaper and hazard free in comparison to chemical insecticides.

Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control (Sadek, 2003). Crude plant extracts often consist of complex mixtures of active compounds, they may show greater overall bioactivity compared to the individual constituents. The deleterious effects of crude plant extracts on insects were manifested in several ways, including toxicity feeding inhibition (Wheeler and Isman, 2001). Neem and Nicotine are usually used as bio-pesticides (Suman Gupta, 2010),

The present study was aimed at The objectives of this experiment wore as follow: To evaluate the role of botanical extracts in the suppression of *H. armigera* in tomato crop, and comparing the efficacy of botanical and biochemical insecticide on qualitative and quantitative properties of tomato fruit.

MATERIALS AND METHODS

Location

The experiment was conducted at the Agriculture Research Station, Baffa (Mansehra) to study the role of some botanical insecticides in control of tomato fruit borer (*H. armigera*) in tomato field during the normal cropping season, 2016. The tomato cultivar Riogrand were chosen for the study because it is liked by the farming communities and grown over vast areas of Pakhal valley in Mansehra.

Experimental layout

The experiments were laid out in randomized complete block design (RCBD with 6 treatments (including check) and three replications. The area of each replicated plot was fixed as 4x3 m². Plot to plot distance were kept as 1 meter to avoid any mixing or drifting of the treatments. Row to row distance was kept as 75cm and plant to plant distance was kept as 30cm. The tomato nursery was raised during February and transplantation was done after three weeks.

Botanical extracts

The botanical extracts and insecticide was utilized and tested as foliar sprays in the experiment. Seed extract of neem (*Azadirachta indica*) was applied at the concentrations of 2%, 5% and 7%, while leaf extract of a weed locally

called pulpuluck (*Persicaria hydropiper*) was applied at the concentrations of 1% and 3% (Jawad *et al.*, 2013). Effects of both the extracts were compared with chemical insecticide having the property of insect growth inhibitor (IGI). The treatments were applied on the occurrence of infestation, which was based on appearance of eggs, larvae or adult of *H. armigera*.

Preparation of the treatments

The biochemical insecticide Match having the property of insect growth regulator was purchased from the local market. Neem seed extract was prepared by soaking one kg of Neem seed in five liters of water and kept undisturbed for 48 h and then ground with an electrical grinder. The crude extract was squeezed out through a muslin and used for preparation of the spray solution. The weed (*Persicaria hydropiper*) was collected locally around the small water channels in the area. One kg weed leaves was blended with blender and filtered through a piece of cloth to get clean and pure extract.

Application of the treatments

The treatments were applied as soon as pest was identified on the plants (Zalucki *et al.*, 1994; Ravi *et al.*, 2008). It was based on observation of any development stages of the pest in the field. The application of treatments was repeated when the pest reached the threshold level (week 4). The crop was visited on weekly basis. Data sampling was done by taking five plants was randomly selected in each replicated plot and tagged for future observations. The post-spray data was recorded after 24hrs, 48hrs, 72hrs and 7days in first week and then in fifth week, further observations were repeated at intervals of one week till crop maturity. At each observation, five tagged plants were checked for presence of larva (e) of *H. armigera*.

Larval population count

The number of larvae on plants per plot was recorded separately. The infestation for each replicated plot were averaged and presented in the form of tables.

Weight and number of sound tomato fruit

The sound tomatoes fruit were collected separately from each plot. Weight and number of standard size tomato fruit was recorded for each replicated plot

separately and was placed as Grade-1. Weight and number of small size tomato fruit were recorded for each replicated plot separately and were placed as Grade-2.

Infested fruit (%)

Weight and number of damaged tomatoes fruit were recorded for each replicated plot separately.

$$\text{Percent fruit damage} = \frac{\text{Number of damage fruit} \times 100}{\text{Total number of fruits}}$$

Yield

The total yield of tomato was determined by adding the produce tomato fruit per picking of the replicated plots in all the treatments. Yield of tomato was taken as g/m², which was then converted to kg/ha by the method followed by Usman *et al.* (2013).

Statistical analysis

Means of all the data was analyzed separately followed by least significant difference (LSD 0.05) with computer software Statistix.

RESULTS AND DISCUSSION

The experiment conducted at the Agriculture Research Station, Baffa (Mansehra) to study the role of some botanical insecticides in control of tomato fruit borer (*H. armigera*) in tomato field during the normal cropping season, 2015.

Table I indicate the number of larvae on tomato crop during the first week of treatment by using 2%, 5% and 7% of the neem extract suppressed the insect significantly compared to the control plots. Results shows that in first 24 h, the highest number of larvae were recorded in weed 1% (0.17) and then similar results were followed by neem 2% and weed 3% (0.13). The least significant larva numbers were noticed in two treatments *i.e.* neem 7% and chemical (0.03). In 48 h the number of highest larva was recorded in Weed 1% (0.2) followed by weed 3% (0.17). The least numbers of larva was noticed in neem 7% (0.03), followed by chemical (0.06) and then two similar treatments that were neem 2%

and 5% (0.13). At 72 h the highest number of larvae were observed in two treatments, weed 1% and weed 3% (0.2). The least significant number of larva was observed again in neem 7% (0.03), followed by chemical (0.1), neem 5% (0.13) and neem 2% (0.17), respectively.

One week data of number of larva was observed highest weed 1% (0.36), followed by weed 3% (0.26) and neem 2% (0.23). The least number of larva was observed in neem 7% (0.06), which was followed by chemical (0.1) and neem 5% (0.13). The mean of number of larvae of overall period (24 h, 48 h, 72 h and 1 week) showed highest in weed 1% (0.23) followed by weed 3% (0.19) and neem 2% (0.17), respectively. The least mean number of larva was observed in neem 7% (0.04), followed by chemical (0.07) and neem 5% (0.12).

TABLE I.- NUMBER OF LARVA IN TOMATO AT DIFFERENT TREATMENTS AT FOUR CONSECUTIVE PERIOD OF 1ST WEEK.

Treatment	24h	48h	72h	7days	Mean
Neem 2%	0.13bc	0.13 bc	0.17b	0.23bcd	0.17bcd
Neem 5%	0.10bc	0.13bc	0.13b	0.13cd	0.12cde
Neem 7%	0.03c	0.03c	0.03b	0.06d	0.04c
Weed 1%	0.17b	0.20	0.26b	0.36b	0.23b
Weed 3%	0.013bc	0.17bc	0.20b	0.26bc	0.19bc
Chemical	0.03c	0.06bc	0.10b	0.10cd	0.07de
Control	0.40a	0.73c	1.13a	1.20a	0.86a

Table 2 indicate the number of larvae on tomato crop during eight week of treatment by using 2%, 5% and 7% of the neem extract, 1% and 5% weed, and chemical that significantly suppressed the insect compared to the control plots. The first week number of larva was highest in weed 1% (0.36), whereas the least was of neem 7% (0.06). In week 2, the highest number of larva was noticed in weed 1% (0.3). These results were followed by weed 3% (0.23) and neem 2% (0.2). The least number of larva was observed in neem 7% (0.07), which was followed by two similar treatments that were neem 5% and chemical (0.13). In week 3, the highest number of larva was recorded in weed 1% (0.4) again similar to that of week 1 and 2. The least number of larva was observed in neem 7% (0.1) followed by chemical (0.17), neem 5% (0.2) and neem 2% (0.23). Similar in 4 week. the highest number of larva was recorded in weed 1% (0.53), followed by neem 2% (0.4) and weed 3% (0.37). The least number of larva was found in treatment of neem 7% (0.13) which was significantly followed by chemical (0.17) and neem 5% (0.27). The highest number of larva in week 5 was noticed

TABLE II.- NUMBER OF LARVA IN TOMATO AT DIFFERENT APPLICATIONS IN OVERALL 8 WEEK PERIOD TIME.

Treatment	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week	Mean
Neem 2%	0.23 bcd	0.20 bcd	0.23 bcd	0.40 bc	0.17 bcd	0.23 bc	0.30 b	0.40 bc	0.27 bcd
Neem 5%	0.13 cd	0.13 cd	0.20 d	0.27 cde	0.13 d	0.17 bc	0.20b	0.23 cd	0.18 cde
Neem 7%	0.06 d	0.07 d	0.10d	0.13 e	0.07 d	0.10 c	0.13 b	0.17 d	0.10 e
Weed 1%	0.36 b	0.30 b	0.40 b	0.53 b	0.27 b	0.30 b	0.33 b	0.47 b	0.37 b
Weed 3%	0.26 bc	0.23 bc	0.30 bc	0.37 bcd	0.23 bc	0.27 bc	0.30 b	0.37 bcd	0.29 bc
Chemical	0.1 cd	0.13 cd	0.17 cd	0.17 de	0.10 cd	0.13 bc	0.17 a	0.20 cd	0.15 de
Control	1.2a	2.10a	2.27a	2.60a	2.93a	3.03a	3.13	3.27a	2.57a

in weed 1% (0.27) followed by weed 3% (0.23). The least significant number of larva was recorded in neem 7% (0.07), followed by chemical (0.1), neem 5% (0.13) and neem 2% (0.17), respectively. The highest number of larva after control (3.03) in week 6 was followed by weed 1% (0.3), weed 3% (0.27) and neem 2% (0.23). The least significant number of larva was observed in neem 7% (0.1), followed by chemical (0.13) and neem 5% (0.17). In week 7, the highest number of larva was recorded in weed 1% (0.33) and then similar in two treatments, neem 2% and weed 3% (0.3). The least number of larva was noticed similar in neem 7% (0.13) which was followed by chemical (0.17) and neem 5% (0.2). In week 8, being the last week, the highest number of larva was observed in weed 1% (0.47), followed by neem 2% (0.4) and weed 3% (0.37), respectively. The least significant number of larva was noticed in neem 7% (0.17) followed by chemical (0.2). Similar the mean number of larva was recorded in control (2.57) which was followed by weed 1% (0.37), whereas the least was noticed in neem 7% (0.1). All the treatments have shown significantly effective control of the larvae compared to the control plots.

Number of larva difference among the different applied treatment to tomato plants is shown in Figure 1. Results show that overall the highest number of larva was observed in control, which was followed by weed 1%. The number of larva raised in start till week 4. after which the number declined evenly in week 5. Much difference cannot be noticed in neem 2% and weed 3%. The least

number of larva throughout the week was observed in neem 7%. In 18, 2nd and 5th week, the number of larva was noticed to be the least whereas the highest number of larva in overall treatments applied was observed in week 8th.

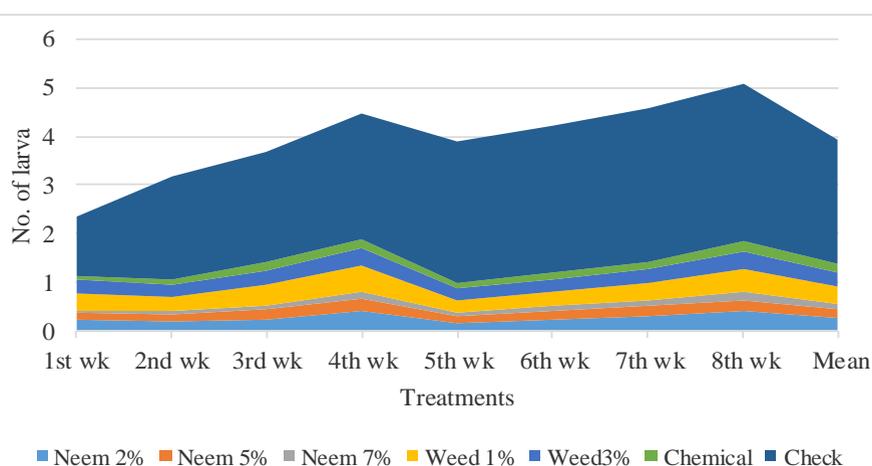


Fig. 1. Effect of different treatments applied to tomato on number of larva in 8 weeks.

Table III indicates the number of larvae on tomato crop during the fifth week of treatment which significantly suppressed the insect compare to control. In first 24 h of 5th week, the highest number of larva was noticed in weed 1% (0.47). These results were followed by two similar number of larva in treatment neem 2% and weed 3% (0.33). The least was number of larva was noticed in neem 7% (0.1), which was followed by chemical (0.16). At 48 h, the highest number was noticed in weed 1% (0.43) and weed 3% (0.33). The least significant number of larva was noticed in neem 7% (0.07), followed by chemical (0.13), neem 5% (0.17) and neem 2% (0.27). The highest number of larva at 72 h was recorded weed 1% (0.33) and weed 3% (0.27). The least number of larva was noticed in neem 7% (0.07) followed by chemical (0.13), neem 5% (0.17) and neem 2% (0.2), respectively. The highest number of larva in 7 day duration was noticed again as in previous weeks. The least significant number of larva was recorded in neem 7% (0.07), which was followed by chemical (0.1), neem 5% (0.13) and neem 2% (0.17). The mean of overall data of number of larva in

various period in 5th week was noticed highest in control (2.78) followed by weed 1% (0.38). The least mean number of larva was observed in neem 7% (0.08) followed by control (0.13) and neem 5% (0.18), respectively.

TABLE III.- THE NUMBER OF LARVAE IN TOMATO DURING DIFFERENT TREATMENTS AT FOUR CONSECUTIVE PERIOD OF 5TH WEEK

Treatment	24h	48h	72h	7days	Mean
Neem 2%	0.33b	0.27bc	0.20bc	0.17b	0.24bc
Neem 5%	0.23b	0.17bc	0.17bc	0.13b	0.18bc
Neem 7%	0.10b	0.07c	0.07c	0.07b	0.08c
Weed 1%	0.47b	0.43b	0.33b	0.27b	0.38b
Weed 3%	0.33b	0.33bc	0.27bc	0.23b	0.29bc
Chemical	0.16b	0.13c	0.13bc	0.10b	0.13bc
Control	2.70a	2.70a	2.77a	2.93a	2.78a

Means followed by different letter (s) are significantly different from each other ($P < 0.05$)

Depending on the difference of the number of larva at different period of week 5th and 1st to various treatment is shown in Figure 2. From the figure it was concluded that in T1 (neem 2%) there was not much difference, however the 7th day number of larva was highest in 1st week compare to 5th week. T2 (neem 5%) showed a slight increase in number of larva of week 5, whereas least difference was noticed in T3 (neem 7%). The highest difference to be noticed was of 7 day in T4 (weed 1%) which was negative, meaning that number of larva in first week was high compare to 5th week. T5 (weed 3%) and T6 (chemical) showed rare increase number of larva in week 5, compare to week 1s! T7 showed the highest positive difference of week 5 compare to week 1, as no treatment was applied, increasing the number larva gradually from start till week 5.

Number of normal and infested tomato fruit response to different treatment is given in Table IV which showed significance efficiency in tomato yield compare to control. The highest number of grade 1 tomato was observed in neem 7% (81) followed by chemical (75.33) and neem 5% (63.67). The least number of grade 1 tomato was observed in weed 1% (43.67), neem 2% (49.33) and weed 3% (51.33), respectively. The highest number of grade 2 tomato was recorded in neem 2% (169.67) which was followed by neem 5% (160.33), neem 7% (153) and chemical (145). The least number of grade 2 tomato was noticed in weed 1% (123.67) and weed 3% (139). Infested tomato per plot was observed highest in weed 1% (91.67) followed by weed 3% (85.33) and neem 2% (82.67). The least number of infested tomato was noticed in neem 7% (54.33), chemical (60) and

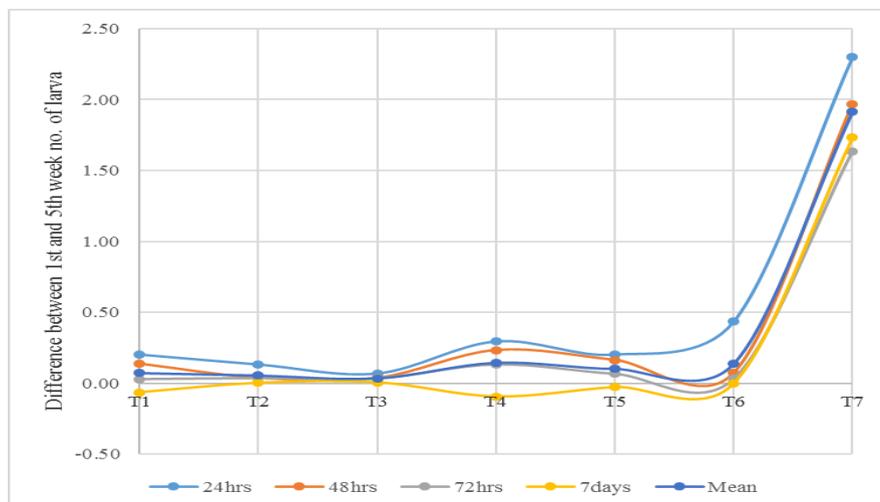


Fig. 2. Effect of different treatments on number of larva in one week duration of 5th week from 1st week.

neem 5% (69.33). Total number of tomato fruit was recorded in neem 2% (301.67) which was followed by neem 5% (293) and neem 7% (289). Least number of tomato per plot was noticed in control (127.33) followed by weed 1% (258), weed 3% (275) and chemical (280.66), respectively. From these results it was concluded that neem extract might have such compounds that help in fruit production as overall neem treatment show slight increase in number of tomato compare to others treatments. Also, with increase in percent of neem extract the number of infested of tomato kept decreasing.

TABLE IV.- RESPONSE TO DIFFERENT TREATMENTS OF NUMBER OF NORMAL AND INFESTED TOMATO FRUIT PER PLOT

Treatment	Grade 1 No. tomato	Grade 2 No. tomato	Infested No. No. tomato	Total No. Tomato
Neem 2%	0.33b	0.27bc	0.20bc	0.17b
Neem 5%	0.23b	0.17bc	0.17bc	0.13b
Neem 7%	0.10b	0.07c	0.07c	0.07b
Weed 1%	0.47b	0.43b	0.33b	0.27b
Weed 3%	0.33b	0.33bc	0.27bc	0.23b
Chemical	0.16b	0.13c	0.13bc	0.10b
Control	2.70a	2.70a	2.77a	2.93a

Means followed by different letter (s) are significantly different from each other ($P < 0.05$)

Weight of grade 1, grade 2 and infested tomato fruit in g/m² per plot followed by kg/ha response to different treatment is given in Table V. Results showed that highest weight of grade 1 tomato was of neem 7% (495.33 g/m²) followed by chemical (484.17 g/m²). The least was noticed in control (73.83 g/m²) which was followed by weed 1% (254.67 g/m²) and neem 2% (296.83 g/m²). The grade 2 tomato fruit per plot was observed highest in neem 2% (559.33 g/m²), followed by neem 5% (545.2 g/m²), neem 7% (324.6 g/m²), chemical (489.83 g/m²) and weed 3% (462.33 g/m²), respectively. The least weight of tomato fruit was recorded in control (242 g/m²) followed by weed 1% (432.67 g/m²). The highest weight of infested tomato was noticed in weed 1% (235.33 g/m²) followed by neem 2% (224.33 g/m²) and weed 3% (221.67 g/m²). The least significant weight of infested tomato was noticed in neem 7% (146.7 g/m²) which was followed by control (154.13 g/m²) and chemical (161.47 g/m²). The total weight of tomato was observed highest in Neem 7% (1019.93 g/m²) whereas the least was of control (315.83 g/m²).

Depending on the number following the weight was determined of infested tomato to different treatment response in Figure 3. The highest percent of infested tomato fruit number and weight was of 17 (control: 37.70% and 32.80%) followed by T4 (weed 1% 35.53% and 25.51%) and TS (weed 3% 30.96% and 22.31%). The least percent of infested number of tomato was observed in T3 (neem 7%: 18.80% and 12.57%) followed by T6 (chemical: 21.38% and 14.22%), T2 (neem 5%: 23.64% and 15.54%) and T1 (neem 2%: 27.4% and 20.76%).

TABLE V.- RESPONSE TO DIFFERENT TREATMENTS ON WEIGHT OF GRADE 1, GRADE 2 AND INFESTED TOMATO FRUIT PER PLOT

Treatment	Grade 1 tomato weight g/m ²	Grade 2 Tomato weight (g/m ²)	Infested tomato weight (g/m ²)	Total tomato weight (g/m ²)	Tomato Weight kg/ha
Neem 2%	296.83c	559.33a	224.33a	856.16b	8561.6
Neem 5%	402.17b	545.20a	174.27b	947.37ab	9473.7
Neem 7%	495.33a	524.60ab	146.70c	1019.93a	10199.3
Weed 1%	254.67d	432.67d	235.33a	687.34d	6873.4
Weed 3%	309.40c	462.33cd	221.67a	771.73c	7717.3
Chemical	484.17a	489.83bc	161.47bc	974ab	9740
Control	73.83e	242.00e	154.13bc	315.83e	3158.3

Means followed by different letter (s) are significantly different from each other (P < 0.05)

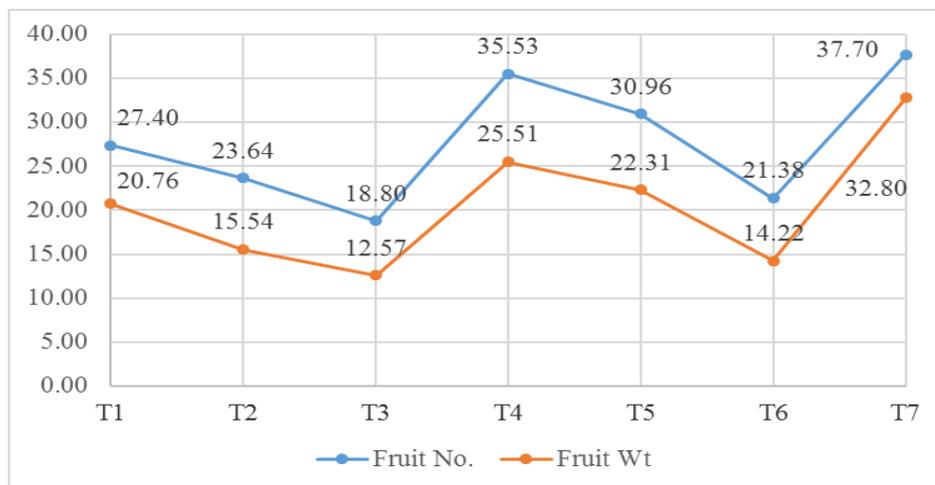


Fig. 3. Effect of different treatments on infested tomato number (%) and weight per plot.

DISCUSSION

The present research results showed that among the various biochemical active crude extract and insecticide, neem extract with 7% had the highest efficacy followed by the insecticide. These also showed the highest in yield tomato fruit numbers as well as tomato weight (g/m²; kg/ha). The results also concluded that till week 4th, number larva increased but then the crude might bioactive vastly, such that the number fell directly in the next week. Further on, the number of larva increased, showing the decrease in efficacy of these treatments.

Guedes and Picanco (2012) determined the manipulation of insecticide into insect growth regulators, also sex pheromone for tomato borer *Tuta* was studied to identify factors of belonging specie in South America. Similar, tomato borer *Tuta absoluta* known for its complete disaster to crop in South America is considered the region from where it spread throughout European and Northern Africa countries (Joel *et al.*, 2010). Moacyr *et al.* (2005) explained integrated pest management (IPM) that spraying twice weekly can give similar productivity and decrease in up to 65.6% pesticide applied throughout the seasons. genes have proven to control insect with inserting gene in host DNA deoxyribonucleic acid), that encode for specific protein providing protection to plant within own physiological system (Mandaokar *et al.*, 2000).

Recognizing the fact that application of pesticide can have no significance increase in yield compare to control, can lead to reduce application of pesticide (Picanco *et al.*, 2007). Plants extract have been discovered to be eco-friendly approach in controlling agriculture pest including *H. armigera* (Kamaraj *et al.*, 2008). Ravi *et al.* (2008) concluded that microbial and neem extract can be best alternatives for sustainable management of tomato fruit borer *H. armigera*. The Neem seed extract have also shown significantly improved tomato plant when applied with combination of *Trichogramma chilonis* (Usman *et al.*, 2012). *H. armigera* also infect chickpea (pod borer), however neem extract have shown maximum efficacy to this crop (Bhushan *et al.*, 2011). In Pakistan, the tomato cultivar being the highest resistance to *H. armigera* is Sahil, Pakit and Nova Mecb (Sajjad *et al.*, 2011).

Neem extract in tomato have been used in management of controlling whiteflies and aphids (Nzanza and Mashela, 2012). The present research results also due in line with the finding of Jawad *et al.* (2013), who studied different botanical extract effect against tomato fruit worm, *Helicoverpa armigera*. Results showed that neem extract treatment had the maximum yield compared to turmeric, henge and garlic extract.

CONCLUSION

Neem extract (5-7%) showed to alter plant resistance against *H. armigera*. Neem 7% showed more efficacy against *H. armigera* compare to local insecticide. Neem (7%) also increased yield in tomato fruit 3% compared to chemical and 20% compared to control.

RECOMMENDATIONS

Among the tested chemicals 7% neem dose yielded good result compared to all tested chemical and proved more effective in suppressing the *Helicoverpa armigera*. Furthermore 7% neem also had increased tomato yield in 20% compared to control plots. Thus keeping in view above results 7% neem is recommended for commercial tomato produced areas.

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**PROF. DR. ROBERTS MERTENS: HIS CONTRIBUTIONS
TO PAKISTAN HERPETOLOGY (1894-1975)**

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Prof. Dr. Roberts Mertens

*Director Emeritus Research Institute, National Science Museum,
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1894-1975*

Prof. Dr. Roberts Mertens, Director Emeritus Research Institute, National Science Museum, Senckenberg, Germany; Honorary Professor at the Johann-Wolfgang-Goethe University, Frankfurt, died on 23rd August 1975, at 81 years of age, of a bite of his pet savanna twig snake *Thelotornis capensis*, while he was feeding it. Prof. Mertens refused treatment and dictated last breaths of life in recording second by second changes occurring in his body as poison worked to stop his heart!

Dr. Mertens had been studying habits of this rare snake by keeping it in a vivarium. Nothing was known about its habits and kind of its venom. Death snatched Professor Mertens from the world of science, and we lost a renowned teacher, researcher, scientist, herpetologist, zoogeographer and a philosopher, leaving a lasting mark on his dedication to science for betterment of humanity!

Son of a fur merchant, Mertens was born in St. Petersburg, Russia, on 1st August 1894, attended local Grammar School, and graduated from University of Leipzig.



Inspired by story of Noah's Arc in school days, and by great collections of specimens from all over the world, shells, skeletons stacked in the shelves of the Senckenberg Museum, made Mertens to realize the enormous manifoldness of life forms in nature, which were systematically arranged by interrelated connections by his teachers Carl Chunus, Heinrich Imrothus and Richard Wolterecks.

Young Mertens decided to take up zoology to work throughout his life, developing special interest in herpetology. He was awarded Ph.D. on his thesis "Investigations on the variability of Italian wall-lizard". During World War I, he served as an interpreter at Bucharest. In 1919, Otto Strassen, then Director of Senckenberg Museum, appointed him as his assistant, where Prof. Mertens stayed for the rest of his life, as Director of the museum. He also lectured in Frankfurt University from 1939-1953.

Dr. Mertens was always helpful to amateurs as well as specialists, received requests from all over the world for identifications and confirmations of amphibians and reptiles. His philosophy has been "if amateurs are properly guided and provided opportunities, can contribute great deal to the universalization of science." He was a stern critic, and would himself hear criticism with open mind.

While working with preserved animals he came to realize importance of study of living animals. He made extensive behavioral studies on living animals in terraria, supplementing his taxonomic studies with ethology and ecology, solving several complex taxonomic problems.

He travelled extensively to gain firsthand knowledge of living animals, he visited Sunda Archipelago, Cameron, Dominican Republic, North America, Australia, Egypt and Siberia. In 1952, he came to Pakistan for two months, collected and observed animals in Sindh and Baluchistan. During these tours he never missed an opportunity to deliver lectures in public.

Pro. He authored several research papers and books on zoology, including *La Vie des Amphibiens et Reptiles* in 1959. An English translation, *The World of Amphibians and Reptiles*, was published in 1960. Mertens described at least 59 reptile species and numerous amphibians. In addition, at least following eight reptile species have been named after Mertens.

Following nine species were named in honor of Dr. Mertens:

Amphisbaena mertensii STRAUCH, 1881
Chalcides mertensi KLAUSEWITZ, 1954
Cryptoblepharus mertensi HORNER, 2007
Erythrolamprus mertensi (ROZE, 1964)
Liolaemus robertmertensi HELLMICH, 1964
Micrurus mertensi SCHMIDT, 1936
Phalotris mertensi (HOGE, 1955)
Phelsuma robertmertensi MEIER, 1980
Varanus mertensi GLAUERT, 1951

CONTRIBUTIONS TO PAKISTAN HERPETOLOGY

Late Professor Dr. Ahsanul Islam (Government College, Lahore) had been in constant contact with Professor Mertens, getting help in identifications etc. for the specimens collected during tours of the Biological Society to northern area or elsewhere in Pakistan. Dr. Mertens was on regular mailing list to receive Journal *Biologia* (Biological Society, Government College, Lahore), thus he had broad knowledge of amphibians and reptiles of Pakistan.

Prof. Mertens based his studies on a large collection of herps from Pakistan deposited in the Senckenberg museum by his associate M. G. Konieczny (Mr. Konieczny a member of oil and gas exploration German team), collected herps from 1959 to 1968 from across Pakistan, and deposited them in the museum. The specimens were studied by Mertens, which formed basis of following series of papers contributing to the herpetology of Pakistan (1959-1974):

- i) 1959. Über einige seltene Eidechsen aus West-Pakistan. *Aqua. Und Terra. Zeit.* 12(10): 307-310.
- ii) 1959. Eine neue Wassernatter aus West Pakistan. *Senckenb. Biol.* 40(3-4): 117-120.
- iii) 1959. Über einige seltene Eidechsen aus West Pakistan. *Aquar. Terr. Zeit.* 12(10): 307-310.
- iv) 1965. Wenig bekannte "Seitenwinder" unter den Wustenottern Asiens. *Natur und Museum*, 59(8): 346-352.
- v) 1969 Die Amphibien und Reptilien West-Pakistans. *Stuttg. Beitr. Naturk.* (197): 1-96.
- vi) 1969. Eine neue Rasse der Dachschildkröte, *Kachuga tecta*. *Senckenb. Biol.* 50: 23-30.
- vii) 1970. Die Amphibien und Reptilien West-Pakistans. 1. Nachtrag. *Stuttg. Beitr. Naturk.* (216): 1-5.
- viii) 1971. Die Amphibien und Reptilien West-Pakistans. 2. Nachtrag. *Senckenb. biol.* 52 (1-2): 7-15.
- ix) 1972. Nachträge zum Krokodil-Katalog der senckenbergischen Sammlungen. 3. Nachtrag. *Senckenb. Biol.* 53: 21-35.
- x) 1974. Die Amphibien und Reptilien West-Pakistans. *Senckenb. Biol.* 55 (1-3): 35-38.

Dr. Mertens described about 61 new species from throughout the world. Professor Mertens described following four new species from Pakistan:

- i) *Enydrus pakistanica* 1959
- ii) *Coluber karelini mintonorum* 1969
- iii) *Echis carinatus astolae* 1970
- iv) *Bufo viridis pseudoraddei* 1991

Professor Dr. Mertens because of his versatile capability and interests held the office of Honorary Chairman of Frankfurt Geographical Society. He was member of:

German Society of Herpetology and Ichthyology, Zoology-Biology Society of Vienna, America Society of Ichthyologists and herpetologists. Indian Academy of Zoology, Nederland Association of Herpetology, Veteran's Society for all Natural Sciences, Herpetologists League, USA, American Museum of Natural History, New York, German Academy of Scientists "Leopoldina".

He founded and edited world's renowned herpetological journal "Salamandra."

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Some Abstracts

PLENARY LECTURES

TURKISH FISHERIES AND AQUACULTURE

Hasan Huseyin Atar

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Turkey is surrounded by four seas, home to several fish species, and giving the country a rich and diverse coastline that supports many activities. Capture fisheries in Turkey employed some 32,599 people and amounted to about 266,078 tonnes in 2014 from all the seas surrounding the country, the Mediterranean, Aegean, and Black Seas, and the Sea of Marmara. The fleet consists of 14,595 vessels. Nearly 5,000 vessels are fishing on the Black Sea, 4,800 on the Aegean while the Sea of Marmara and the Mediterranean have 2,700 and 2,095 respectively. The distribution of catches from each water body is far from uniform. The Black Sea is responsible for approximately three fourths of the annual catches, followed by the Sea of Marmara with about 10%, and the Aegean with 8%. The main species in terms of volume are the small pelagics, anchovies, pilchards, sprats, and horse mackerel which amounted to 73% of the catch in 2014 and which are caught primarily in the Black Sea. They are used almost exclusively for the production of fishmeal and fishoil, two of the main ingredients in fish feed. Fish for human consumption on the other hand comes from all the seas surrounding Turkey though the Black Sea catches are significantly higher than those from the other three. Turkey has a dynamic marine aquaculture industry located primarily on the Aegean Sea coast and employing some 8,000 people. Marine fish farming increased from 88.600 t in 2010 to 100.900 t in 2012. The number of vertically integrated groups operating their own hatcheries, fish feed plants, fish farms, and processing and packaging facilities is increasing constantly. The main species farmed in 2014 here are seabass (74,653 tonnes) and seabream (41,873 tonnes). The Black Sea is the site for a modest production of sea-raised trout (4,812 tonnes). There is also a large and growing production of freshwater rainbow trout (107,983 tonnes) in the interior of the country. Turkey is ranked first in the Mediterranean for seabass farming and second, behind Greece, for seabream. It has a 25% market share of the seabass and seabream trade in Europe.

CONSERVATION AND WISE USE OF AQUATIC AND TERRESTRIAL ANIMALS

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According to the Theory of Evolution by Charles Darwin (1809-1882), new species of animals and plants, evolve continuously. Therefore, we do not know how many species of animals and plants exist on Earth. Scientists believe there can be 50-100 million species. Out of which only about 1.5 million species have been reported or described. About 10,000 new species of animals are discovered annually in various parts of the world. Therefore, there are plenty of rooms for research on taxonomy, biology and other aspects including their exploitation for human welfare. Some tiny creatures may emerge as instrumental in addressing our food security, income generation and employment creation for which the whole world is striving. Animals are good sources of protein with rich profiles of amino acids. Demand for animal protein is rapidly increasing due to increase in population and income. However, very few numbers of species have been used so far to meet our

animal protein need. We consume annually approximately 130 million metric ton (mmt) of fish and aquatic animals, 114 mmt of pork, 106 mmt of chicken, 70 mmt of eggs, 68 mmt of beef, 14 mmt of lamb and some other meat. One way of increasing animal protein supply is to increase productivity of currently farmed animals through improving in genetics, biotechnology, feeding and other management. Another way is to explore potential new species for farming. Recently FAO has highlighted the potential of farming insects for human food because they grow fast and easy to breed. There are nearly 1 million insect species but only a few of them are traditionally consumed by some ethnic groups in rural areas. We know and farm only few species of birds such as chicken, duck, turkey and few others out of 10,000 species found on earth. Similarly, we farm only handful of terrestrial animals e.g. cattle, buffalo, goat, sheep, rabbit, etc. out of 5,000 mammals. Similarly, out of 30,000 aquatic species, we farm only about 500. Very few species have been domesticated for human consumption out of more than 8,000 reptiles and 6,000 amphibians. People hardly know much about 80,000 mollusks and 40,000 crustaceans although they are abundant in the nature; snails, oysters, mussels, crab, crawfish, lobster, shrimp and prawn. Scientists believe mass extinction of species happened five times in the history of earth formation at different times millions of years ago due to either volcanic activity, climate change and so on. Now 6th mass destruction is progressing. According to IUCN, over 16,000 species on earth are threatened and 10-25 species are extinct per year. Habitat destruction, climate change and global warming, pollution, hunting or over fishing, and predation by other animals are the major factors behind the extinction of the species. Human activities e.g. commercial farming, building roads, dams, mining, producing fuel and gases and pipelines, tourism, manufacturing factories etc. have been blamed. Every minute nearly 20 ha of forest is destroyed affecting wild animals. Annually, 8 million tons of plastics reach to the sea each year, which kills the endangered sea turtles. We need to change our living style to save the animals and the planet. Value of saving species can be understood from the fact that honeybees in the US, which are threatened species in many parts of the world, pollinate the crops, which worth over US\$10 billion. More facts and figures will be presented during the congress.

LEADING ROLE OF BIOLOGISTS FOR THE DEVELOPMENT OF BIOFUEL SECTOR IN PAKISTAN

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Fossil fuels' increasing prices and resource depletion have necessitated the search for sustainable supplies of alternatives fuels. Biofuels have additional advantage of being environment friendly with no generation of recalcitrant pollutants. Food competing biomass derived feedstocks such as starch and sugars cannot be afforded for scalable fermentations of biofuels. Whereas lignocellulosic biomass, the largest renewable feedstock is abundantly present throughout the biosphere. The photoautotrophically regenerated cellulosic biomass represents the first level of bioenergy dynamics at which solar energy is trapped into the form of complex polysaccharides; which can then be depolymerized and used as energy liberating molecules. The complex plant biomass had been serving as energy (food) source to the second and onward trophic levels. However, large reservoirs of lignocellulosic biomass become solid wastes, whose natural decomposition and circulation is relatively a slow process. The recalcitrant nature of lignocellulosic feedstocks has now been knocked by chemists, biochemists and biotechnologists for its destructuring and making the cellulosic and hemicellulosic fractions available for chemical or

enzymatic hydrolyses to liberate streams of hexoses and pentoses. Both categories of the sugars can then be fermented to ethanol, hydrogen and other biofuels, which can be used as transport fuel or to fuel industries and domestic needs. At present the lignofuels are not cost as well as energy competitive with fossil fuels. But the bioenergy sector is to be developed before the complete exhaustion of fossil fuels. The biologists can design the strategies for economical and ecofriendly pretreatments, hydrolyses and fermentation of diverse of lignocellulosic feedstocks with full utilization of ferment residues as soil conditioner/fertilizer and animal feed supplement. Biologists in Pakistan can focus research to identify the best feedstocks or combination of feedstocks which can yield promising results in a given habitat while applying specific microbe(s) for the feasible generation of biofuels and additional valuable chemicals. In this lecture, potential role of biologists at different levels of second and third generations biofuels' productions will be discussed.

**DEFEATING DEADLY DISEASES BY SENSITIZING COMMUNITIES: THE ROLE
UNIVERSITIES CAN PLAY IN SAVING LIVES OF FELLOW COUNTRY MEN**

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Hepatitis B, C and HIV/ AIDS has become the greatest disaster among infectious diseases throughout the world. These diseases particularly victimize the developing world. These diseases cause a multi faceted damage both to the lives and economy of affected people and states. Almost one in ten Pakistanis is suffering from one of these ailments. Being a low income country, Pakistan has been under the attack of these curses which are inflicting the mortality of over a million people annually as well as multi-billion dollar drainage of capital from the country in order to manage available therapeutic options. In order to halt the devastations of Hepatitis B and C, a unique cost effective project SATH, was launched by the University of AJK in Collaboration with Rahma Islamic Relief and the Department of Health Govt of AJK at the university of AJ&K. During the project the total population of University of AJK was screened for hepatitis B and C. All those individuals who appeared positive in the initial screening were diagnosed properly for the presence of respective virus by using Polymerase Chain Reaction. The patients appearing positive after complete diagnostics were treated for the respective disease free of cost. The awareness component of the project was focused on raising awareness among the students and then towards the communities those students belonged to, targeting over one million populations of peripheral communities. a total of 7015 students, teachers and employees of the University of AJ&K were screened for hepatitis B and C. In the initial screening a total of 182 cases were found positive for either hepatitis B (115) and (or) C (67). After the complete diagnostics 126 cases were found to be carrying the viral genomes in their blood. The treatment of the positive cases has been underway, and by the end of December 2016 a total of 15 cases had been found to be cured of hepatitis C, while 2 of HBV cases have been reported negative after treatment. During the awareness raising campaign against hepatitis, almost 350,000 individuals across the state of AJK and some districts of Pakistan had been sensitized by the end of December 2016. The awareness raising was done from grass root levels at barbers shops, educational institutions, Religious Madaris, Majalis Aza and Journalists clubs to the highest levels of the Government Officials, Policy Makers, Members of Parliament and the Ministers of State Machinery. The awareness campaign has paved way for proper measures in eradication of deadly hepatitis and AIDS, taking all stake holders on board. A very cost effective integrated approach to defeat deadly diseases adopted by the University of AJ&K has been a tremendous effort resulting in the life saving of over 350,000 individuals with

creating a number of research opportunities for the researchers of the University. Resources were generated from unconventional sources and a pay back approach was inculcated among the university students and faculty members. This is a worth adopting research and relief model for rest of institutions of the country in particular and whole of the developing world in general.

ALTERNATIVES TO ANTIBIOTIC AS FEED ADDITIVES

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Antibiotics have been used for decades to promote health, growth and production of farm animals. While their therapeutic role is still recognised, their prophylactic use especially in food animal diets is not acceptable anymore. This is due to the increasing evidence of resistance of commonly occurring infections against previously effective antibiotics in humans. The increased antibiotic resistance is perceived to be associated with the consumption of food being derived from animals that receive antibiotics in their diets. This caused a great concern for the public and policy makers which resulted in the ban of prophylactic use of antibiotics in food animals. This ban has created concerns amongst the animal industry practitioners who have observed the benefits of using antibiotics to maintain health and vitality of animals. Therefore there is a need to develop alternatives which not only replace the use of antibiotics as growth promoters but also promote gut health, feed efficiency and environmental targets for the animal industries worldwide. This presentation explores a few of the options that can be used in the form of plants, herbs and their extracts to optimise digestive efficiency and performance of animals. The ultimate goal is to develop alternative additives which boost animal health, improve production and enhance the quality of animal derived foods for their safe use in human diets.

STUDY OF SOME ANTHROPOGENIC NEGATIVE IMPACTS ON THE WETLAND ECOLOGY OF BIRDS IN THE PUNJAB PROVINCE OF PAKISTAN

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Studies were conducted on some anthropogenic negative impacts on the ecology of the wetland birds, mainly the wintering migrants, at four barrages of the rivers and a lake in the Salt Range of Punjab province of Pakistan, from autumn 2004 to spring 2016, in 4-6 field trips of 3-4 days each. The study areas were at the barrages of Balloki at river Ravi, Qadirabad at river Chenab, Rasool at river Jhelum, Chashma at river Indus and Kallar Kahar lake in Salt Range. The habitats of all these barrages are mostly similar, which comprise of marginal protection 'bunds' on both sides of the rivers, upstream of the barrage and spur 'bunds' projecting from the protection 'bunds', inwards towards the river. There are permanent seepage or floodwater ponds on both sides of these bunds, created due to the excavation of earth for these bunds. The water level of the ponds on the river side of the marginal bunds fluctuates with the closing or opening of the gates of the barrages. These ponds also get flooded when rivers get inflated during the flood seasons. There is always thick growth of tall water reeds and bulrushes in shallow water and on the margins of these ponds.

The floating vegetation is commonly Hyacinths and Nelumbo. *Trapa* sp. water nuts, is also a floating plant, that grows naturally or cultivated in some ponds. Bottom flora is commonly the *Hydrilla* and *Potamogeton*. These ponds have abundance of phytoplankton, zooplankton, macroinvertebrates and vertebrates, particularly the stocked fish. The ponds or water reservoirs of the barrages are suitable habitats for birds for feeding, breeding and for roosting. During the winters huge influxes of migratory water birds visit these habitats and remain in open water at least for the whole day. Millions of migratory passerine birds also roost for the night in the tall marginal vegetation of the ponds. Chashma Barrage is special, as it has huge water reservoirs, several kilometers long and wide on its both sides upstream of the barrage. These are connected to the main river on one side. The storage water is used for a hydropower station and to feed Chashma-Jhelum link canal, during the less flow of water in the river, usually in winter. These water reservoirs are staging or wintering places for the huge influxes of migratory birds travelling along the Indus flyway. Kallar Kahar Lake is a large but shallow, brackish water wetland, surrounded by hills on three sides. It is very much clogged with submerged flora. Its periphery and the areas where water is very shallow, are grown with semi submerged water reeds. This growth shelters a good number of gallinules, herons, egrets, and migratory water fowl in winter. Thousands of black starlings also roost for the night in this tall growth. During autumn transit migration, many thousand wagtails, pipits and sand martins also roost for the night in the lake's thick and tall vegetation growth. Tourism of this lake, throughout the year, causes disturbance for the birds. The lake is threatened due to increasing urbanization around it. There are hotels, restaurants and shops at the edge of the lake and still more are being made. The hill slopes are being terraced for more constructions. Even residential housing colonies are coming up around the lake. As a result of these, during the heavy precipitation of monsoon season, large scale sediments are washed down to the lake. There is sewage discharge from the houses to the lake. In addition to that all houses have shallow pumps, which have resulted in over pumping and induced depletion of the lake, which is causing eutrophication and damage to the ecosystem. The quality of lake water has exceeded the NEQS limits. The water of the reservoirs/ponds of all these wetlands, are rich in nutrients due to the droppings of the huge influxes of the wintering waterfowl in the water during their diurnal presence and the passerine birds in millions, for the nocturnal roosting in the water edge vegetation. These nutrients, such as Nitrates and phosphates were measured as rich, when compared with the non migratory birds seasons. These wetlands have luxurious growth of bottom flora, suspended and floating plants and plankton. That resulted in rich aquatic biodiversity, which boosted not only high yield of fish, but also attracted large number of piscivorous birds, which play healthy role for fish production. The habitats of all these wetlands are constantly being commercially exploited, which have negatively impacted the avian fauna, both sedentary and migratory. It was observed that the diurnal roosting, feeding and preening of the large flocks of ducks and coots was disturbed and reduced in those habitats where marginal vegetation was commercially exploited. It was experienced during the study period that the millions of the passerine birds that roosted for the nights, every year, in the marginal vegetation, of Balloki wetlands, ceased coming to these over exploited habitats. The marginal flora of river Ravi ponds that was badly over exploited commercially and its water remained polluted with chemical toxins, is almost no more visited by the winter bird migrants. Even the resident purple gallinules have vanished. At Chashma Barrage over 100,000 grebes, cormorants, ducks and coots were counted in one day, floating in one of the large water reservoirs and over 2,000 tern and gulls were estimated flying low and diving for small surfacing fish in the same reservoir. Among the waterfowl there were diving ducks which often dived to graze the bottom flora and there were also the dabbling ducks which rested for the day in the middle of the reservoirs. In the evening, the dabbling ducks fly to shallow edges of the water of the reservoirs to feed on plankton, avoiding the human disturbed areas. After their night feeding,

the daphnia and copepod numbers reduced considerably from the puddles and shallow edge water, as compared to the disturbed area of the same habitat. That resulted in less consumption of dissolved oxygen, which obviously benefitted tiny fish. At Qadirabad and Rasool Barrages' negative impacts of the habitat degradation were noted on the ethology of the Marsh Harriers, Egrets and black starling. These birds have communal night roosting, for which they have circle flights in wait for other birds to join, late in the evening and then roost communally into the bulrushes and water reed thickets. Increasing commercial exploitation of the habitats is hampering the wintering birds' night roosting.

RECOMMENDATIONS

1. Wetlands at the barrages need further studies on the socioeconomic impacts on the ecolinkages of birds.
2. Each wetland needs a management plan and its coordinated implementation.

SECTION - I

CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS,
PHYSIOLOGY, TOXICOLOGY

1. BIOCHEMISTRY, BIOTECHNOLOGY

DEVELOPMENT OF MOLECULAR TOOLS FOR THE DIAGNOSIS OF *PLASMODIUM VIVAX* AND *PLASMODIUM FALCIPARUM* USING CYTOCHROME C OXIDASE GENE

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Pakistan has been facing extreme challenges to curb **Plasmodium vivax** and **Plasmodium falciparum** which are creating lots of trouble across the country. Accurate and prompt diagnosis of infectious disease is crucial for reducing malaria burden. Traditional gold standard microscopic detection often give less reliable results in mixed infection or in low grade parasitemia. PCR based methods using DNA are more sensitive and specific tools to detect malaria parasites. Some PCR assays, although sensitive yet need multiple reactions to detect mixed infections or species identification making it time consuming and expensive. The present study aimed to target mitochondrial genome of Plasmodium species which are species sensitive using one step PCR assay to get more accurate detection. Primers were designed to detect **Plasmodium vivax** and **Plasmodium falciparum** in malaria positive blood samples which are collected during May–October 2014. A total of 81/130 samples were slide positive for Plasmodium. From which 78 samples were positive for *P. vivax* and 3 samples were *P. falciparum* positive. While no mixed infection found during analysis of blood slides. These samples were analyzed using cox I and cox III mitochondrial genes species specific primers. Out of 130 total blood samples, 83 were malaria positive from which 75 (90.36%) samples were *P. vivax* positive while rest 7 (8.44%) were *P. falciparum* positive using PCR. One patient was found with mixed infection. Compared to microscopic results, for *P. vivax* and *P. falciparum* the sensitivity of the PCR targeting mtDNA amplification was higher

EFFECT OF VARIOUS LEVELS OF IRON ON PEROXIDASE ACTIVITY IN THE FISH,
CIRRHINA MRIGALA

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Present research work was conducted to evaluate the effect of various levels of iron on peroxidase activity in the fish, *Cirrhina mrigala*. For this purpose, four groups of *Cirrhina mrigala* (one-year old) were exposed to different treatments viz. 96-hr LC₅₀, 2/3rd, 1/4th and 1/5th of LC₅₀

concentrations of iron for 30 days in the glass aquaria with three replications for each treatment. Activity of peroxidase in the gills, liver, kidney and brain was assessed by measuring the conversion of guaiacol to tetraguaiacol, spectrophotometrically, at a wavelength of 470nm. The results reveal that peroxidase activity was increased significantly ($p < 0.01$) in gills, liver, kidney and brain after exposure of iron as compared to the control group. Peroxidase activity of 0.697 ± 0.005 , 0.584 ± 0.003 , 0.456 ± 0.002 and 0.287 ± 0.004 U mL^{-1} was recorded in the metal stressed fish gills, liver, kidney and brain, respectively while in control fish, the same for all the organs was observed as 0.119 ± 0.003 , 0.111 ± 0.005 , 0.107 ± 0.005 and 0.024 ± 0.005 U mL^{-1} , respectively. Physico-chemical parameters viz. pH, dissolved oxygen, carbon dioxide, total ammonia, total hardness, calcium and magnesium of the test media were monitored daily. All the physico-chemical variables of the test media varied significantly at $p < 0.01$. All these variables exerted significantly positive effect on peroxidase activity except dissolved oxygen in both the organs.

PROXIMATE PARAMETRIC AND MINERALS COMPOSITION OF RAW CATTLE MILK FROM THREE DISTRICT OF SOUTHERN PUNJAB, PAKISTAN

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Eighty seven cattle raw milk samples were collected from three districts of southern Punjab to evaluate the variation in chemical composition of milk as affected by location by location and parity. Following proximate and mineral parameters were evaluated: total solids (TS), fat, solid-not fat (SNF), lactose, crude protein (CP), total ash (TA), major (calcium sodium (Na), potassium (K) and minor minerals (iron (Fe), zinc (Zn), and nickel (Ni)). The average values and their significance for different parameters are listed here under: TS ($12.1 \pm 0.38\%$) differed with location ($P < 0.05$) but did not differ among parity (> 0.05), fat ($3.9 \pm 0.29\%$) did not differ with location or parity ($P > 0.05$). SNF ($2 \pm 0.35\%$) differed among location ($P < 0.05$) but did not differ with parity ($P > 0.05$), CP ($3.63 \pm 0.11\%$) did not differ with location but differed with parity ($P < 0.05$). TA ($0.63 \pm 0.01\%$) significantly affected by parity ($P < 0.05$), lactose ($3.8 \pm 0.36\%$) differ among location ($P < 0.05$) but did not differ with parity ($P > 0.05$), Ca (465.12 ± 110.69 ppm) did not differ with parity and location ($P > 0.05$), Na (17.76 ± 4.361 ppm) and K (58.97 ± 15.22 ppm) did not differ with both location and parity ($P > 0.05$), Zn (0.29 ± 0.013 ppm), Fe (0.32 ± 0.049 ppm) differed significantly with parity ($P < 0.05$), Ni (0.32 ± 0.049 ppm) did not differ with both location and parity ($P > 0.05$). It is concluded that locality of the lactating animal causes to change the proximate composition of major nutrients of raw milk. Also, the no. of parity does not affect the concentration of most minerals except Zn and Fe.

**CONCENTRATION BASED EFFECT OF ZINC ON PEROXIDASE
ACTIVITY OF *CATLA CATLA***

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Antioxidant enzymes are the first line of defense against reactive oxygen species (ROS) and other free radicals. When the rate of ROS exceeds the capacity of antioxidant enzymes, their non-detoxified radicals begin to attack the bio-molecules. To check the concentration based effect of zinc, *Catla catla* were exposed, separately, to 96-hr LC₅₀ of zinc and its sub-lethal concentrations viz. 2/3rd, 1/4th and 1/5th of LC₅₀ for 30 days in glass aquaria at constant laboratory conditions. After 30-day, the fish were sacrificed and their liver and kidney analyzed for peroxidase enzyme activity. Change in activity of peroxidase enzyme in the fish exposed to sub-lethal concentrations was compared with the control. The data were subjected to statistical analysis by following Factorial design. Dose dependent increase in the activities of peroxidase enzyme was observed in the tissues of fish as compared to the control group. There was a significant difference between the peroxidase activities in metal stressed fish and control group. Peroxidase activity in the liver of fish from all the treatments was significantly ($p < 0.05$) higher than that of kidney. The results of these studies in fish tissues may prove that peroxidase activity can be used as a sensitive bioindicator of the antioxidant defense system.

**EFFECTS OF SUB-LETHAL DOSES OF ZINC CHLORIDE ON PEROXIDASE
ACTIVITY IN *CIRRHINA MIRIGALA***

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Effect of sub-lethal doses of zinc chloride exposure on peroxidase activity in the kidney and liver of *Cirrhina mrigala* was observed. For this purpose, three groups of *Cirrhina mrigala* (one-year old) were exposed, separately, to sub-lethal concentrations (2/3rd, 1/4th and 1/5th) of 96-hr LC₅₀ of ZnCl₂ in glass aquaria along with control group. After 30 days exposure, the fish were sacrificed and peroxidase activity in liver and kidney were determined and compared with that of control. Physico-chemical parameters of the experimental media viz. pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium and total ammonia were monitored twice a day. The enzyme peroxidase activity was significantly increased in both the organs as a result of zinc chloride exposure as compared to control, while among the organs, the peroxidase activities in all the treated and control fish groups were found lower in kidney than in the liver. Statistically significant ($P < 0.05$) and positive dependence of peroxidase activity in the treated fish liver and kidney was observed for the selected physico-chemical variables, except dissolved oxygen.

EGG QUALITY AND ORGANOLEPTIC EVALUATION OF SIX CHICKEN BREEDS IN PAKISTAN

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The study was conducted with the aim to compare egg quality and organoleptic evaluation of different chicken breeds (Fayoumi, White Leg horn, Aseel, Rhode Island Red, Naked Neck and White Plymouth Rock). For egg geometry and quality analysis, 10 egg samples of each breed were randomly taken from Indigenous Chicken Genetic Resource Center (ICGRC), University of Veterinary and Animal Sciences, Ravi Campus, Pattoki. For sensory evaluation, all eggs were boiled at a time under Completely Randomized Design. A semi trained panel of 20 people evaluated three egg samples from each breed on 15 points hedonic scale. The data thus collected were analyzed through one way ANOVA and significant means were compared through Duncan's Multiple Range test using SAS 9.4. Eggs of Fayoumi breed showed higher specific gravity, Haugh unit score, yolk color, and yolk percentage. Similarly, Rhode Island Red had higher albumen percentage, while yolk percentage was highest in Naked Neck eggs. All sensory attributes were found highest in eggs of White Leghorn except albumen color which was poor in Aseel eggs. It can be concluded that Fayoumi have better internal egg quality and leghorn eggs have better organoleptic evaluation.

PROXIMATE AND MACRO MINERALS COMPOSITION OF EGGS FROM SIX CHICKEN BREEDS IN PAKISTAN

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A study was conducted to compare egg proximate composition and macro minerals profile of six different chicken breeds {Fayoumi, White Leg horn (WLH), Aseel, Rhode Island Red (RIR), Naked Neck (NN), and White Plymouth Rock (WPR)} in Pakistan. For this purpose, 5 birds (40-50 weeks of age) of each breed were kept on litter floor under Completely Randomized Design. A total of 10 eggs from each breed were used for proximate analysis (Dry matter %, Crude protein %, Crude Fat %, Ash%, Carbohydrates %) and 3 from each breed for determining macro minerals (Iron, Magnesium, Potassium, Calcium, Sodium). For data analysis, Analysis of Variance (ANOVA) technique was used and significant means were compared with Duncan's Multiple Range (DMR) test using SAS 9.4. Results showed significantly highest moisture and carbohydrates percentage in eggs of WLH, while, eggs of Aseel, NN, and WPR had highest protein percentage as compared to other breeds. Moreover, eggs of WPR also had more lipid contents as compared to other breeds. However, Ash percentage remained comparable among different breeds. Minerals analysis showed significantly higher Iron percentage in eggs of WLH and RIR, Magnesium in Fayoumi and WPR, Potassium in NN and Fayoumi, Calcium in NN, and Sodium in Fayoumi. It can be concluded that genetic differences do affect egg composition.

SEASONAL VARIATIONS IN HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILE OF *CHANNA MARULIUS* ARE COMPLEMENTARY TO THE CHANGES IN WATER QUALITY PARAMETERS OF RIVER CHENAB IN PAKISTAN

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The river Chenab is a rich source of biodiversity and 33 fish species has been identified and reported from Chenab waters including *C. marulius*. The giant snakehead, *C. marulius* (Hamilton), is a fast growing fish having high market value and consumer preference in Pakistan. The aim of this study was to determine the seasonal variations in hematological and selected parameters of serum biochemical profile of *C. marulius* and to correlate them with the changes in water quality parameters of River Chenab during the two consecutive sampling sessions. Water and fish samples were collected from the same pit area of Muhammad Wala Head works during December to April for the seasons 2010-11 and 2011-12 respectively. A total of 51 *C. marulius* specimens (33 during first and 18 during second sampling season) were captured with the help of fishing nets. Water quality parameters and complete blood count for *C. marulius* was determined by standard protocols. Our results indicated significant variations in hematological parameters of *C. marulius* during both sampling seasons that were complementary to the changes in water quality parameters of river Chenab indicating that environmental changes affects the blood chemistry of *C. marulius*.

USE OF CYTOCHROME C OXIDASE1 (CO1) GENE IN MITOCHONDRIAL DNA AS A TOOL FOR FISH SPECIES IDENTIFICATION

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Fish species identification has been traditionally carried out on visible morphology. There are some limitations found in this type of identification. For example, visible morphology does not identify the organism at its developmental stages and when a damaged specimen is under consideration due to incomplete morphology. These kinds of limitations present in morphometric system raised the need for new method of identification. DNA taxonomy has been recently proposed as solutions to the crisis of taxonomy. Mitochondrial DNA-based methods have several advantages because DNA is less sensitive to degradation and can be accessed in all stages from egg to adult. The focus is on mitochondrial cytochrome c oxidase 1 (COI) unit because it appears to be among the most conservative protein coding genes in the mitochondrial genome of animals. COI power to discriminate closely related species is largely attributable to the abundance of synonymous nucleotide changes. During present project three fish species from River Ravi, and River Satluj were identified morphologically at the beginning of study and then genetically. During genetic identification DNA was extracted from fins of fishes and Polymerase Chain Reaction for amplification of short sequence of COI gene was performed with specific primers and temperature conditions. PCR products with gene of interest were sequenced and analyzed by using BLAST and MEGA7. Results showed that genetically identified fishes were same as were identified at the beginning of study. These results indicate that COI gene is a useful marker for

species identification and can be used for identification of fishes and also for the construction of a reference library of Pakistan fish fauna.

MOLECULAR BASED IDENTIFICATION OF AN ENDEMIC FRESH WATER FISH SPECIES FROM RIVER RAVI IN PUNJAB, PAKISTAN

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Taxonomy has always been a challenging job for the scientists. Traditional method for identification of fishes is based on morphological features which sometimes create great confusion due to great similarity in closed species. DNA barcoding is a species identification method in which a short standardized mitochondrial DNA region is used. It has received much attention recently, and is being further developed through an international initiative. In this method sequence diversity in a 658 base pair fragment near the 5' end of the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene is employed as a tool for species identification. This method is capable enough for identification of processed, preserved and damaged fishes as compared to traditional morphometric method and is also appropriate for rapid identification of juveniles as well as adult stages of fishes. Present study was conducted to identify *Mystus cavasius* on molecular bases. This fish is commonly known as Kinger fish. This is endemic fresh water fish of Punjab, Pakistan. Partial sequence of CO1 gene was amplified by using conventional PCR method. PCR products with gene of interest were sent for sequencing. Sequencing results were analyzed by using different soft wares. Analysis confirmed that genetically identified fish was the same fish which was identified morphologically at the beginning of the study. The phylogenetic tree drawn through MEGA 7, revealed the evolutionary distances between *Mystus cavasius* and other fish species from different genus and families. From these findings, it can be concluded that the partial CO1 gene sequence, may serve as milestone for identification of related species at molecular-level.

CLINCO-HEMATOLOGICAL AND SOME SERUM BIOCHEMICAL CHANGES INDUCED BY TRIAZOPHOS (ORGANOPHOSPHATE) IN COMMON CARP (*CYPRINUS CARPIO*)

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This study was conducted to observe the clinco-hematological and some serum biochemical effects induced by triazophos in fish *Cyprinus carpio*. For this purpose 30 fresh water fishes were

purchased from fisheries complex Bahawalpur and transferred in laboratory of department of life sciences The Islamia University of Bahawalpur. The fishes were divided in to five groups in glass aquaria named as A, B, C, D and E. Group A is consider as control group all other groups were experimental groups. Triazophos applied @ 00, 0.25, 0.50, 1.00, 1.30 mg/L to all groups respectively for 96 hours. Blood was collected after 72 and 96 hours. Fishes in treated groups show different abnormal clinical signs. Absolute and relative weight of different body organs was significantly decreased as compared to control group. Hematological parameters hemoglobin, total erythrocyte count, pack cell volume, mean corpuscular hemoglobin concentration and lymphocytes were significantly decreases as compared to control group A while total leucocyte count, mean corpuscular volume and neutrophils were significantly increased when compared to control group. Serological parameters urea, cretenine, ALT, AST, CK-MB, CPK and serum glucose were significantly increased when compared to control group. The frequency of different nuclear and cellular parameters was also increased. Hence it is concluded that triazophos induced clinic hematological and some serum biochemical changes including nuclear and cellular abnormalities in fish *Cyprinus carpio* when treated with different concentrations of triazophos.

A NOTE ON THE BIOCOMPATIBILITY OF ZINC OXIDE NANOPARTICLES IN MALE ALBINO MICE

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The present study was conducted to account for the synthesis, characterization and biocompatibility of zinc oxide nanoparticles (ZnO NPs) in adult male albino mice. ZnO NPs were synthesized by the sol-gel auto-combustion method and then characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and tunneling electron microscopy (TEM). The XRD confirmed the formation of single phase ZnO. The average particle size is in between the range of 40 to 55nm as revealed by SEM and TEM. Ten week old male albino mice were divided into four experimental groups; A, B and C were orally treated with 50 (low dose), 300 (medium dose) and 600mg/ml solvent/ kg body weight (high dose) of ZnO NPs for four days. The serum biochemical parameters, toxicity responses and histological changes in mice were determined in all four experimental treatments. Results indicated significantly higher Bilirubin levels in mice exposed to medium (p =0.01) and high doses (p= 0.002) of ZnO NPs as compared to control group. ALT concentrations were significantly lower (p = 0.05) while creatinine was significantly higher (p = 0.04) in mice exposed to low and high dose of NPs. Histological analysis of liver and kidney in four treatments revealed a dose dependent effect of NPs on anatomy of studied vital organs. It was observed that oral treatment of 50 mg/ml solvent /kg body weight of ZnO NPs did not result in any obvious health hazard in subjects. In contrast, the higher doses have reasoned toxicity in the subject animals. Based on our results, It is recommended that ZnO NPs should be used at less than 50 mg/ml solvent /kg body weight concentrations in commercial products.

EFFECT OF POLYMORPHISM IN MYF5 WITH MEAT QUALITY TRAIT IN CATTLE

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The growth rate is the most important trait which affect the meat quality in Pakistani cattle. MYF5 gene has major role in the growth and development of the bovine. It is associated in the differentiation of skeleton muscles. We examined the polymorphism SNP in the exon of MYF5 gene by using PCR-RFLP. The SNP marker is associated with the genotype of MYF5 gene. We determined the novel SNP at the 6574 bp in exon 3 of bovine MYF5 gene. The C→T mutation was Miss-sense mutation which made a change from serine (TCT) to Phenylalanine (TTT). It is analyzed that the gene specific SNP marker is influence with MYF5 genotype and showed the effect on the weight of bovine. The results suggested that the MYF5 has a close linkage with the Quality trait loci (QTL) affecting the meat quality trait in the bovine. The gene specific markers in MYF5 may be useful for meat quality and growth trait in Pakistani cattle breeds.

DIETARY REQUIREMENTS OF MYO-INOSITOL FOR *LABEO ROHITA* FINGERLINGS FED PRACTICAL DIET

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Present research project was designed to determine the dietary myo-inositol requirement for *Labeo rohita* fingerlings. Five experimental diets namely DI, D2, D3, D4 and D5 were formulated by supplementing myo-inositol at the level of 0, 100, 200, 400 and 800 mg/kg. Fifteen fingerlings (4.3±0.07 g/fish) were stocked in duplicate tanks against each experimental diet. Fingerlings were fed once a day to apparent satiation for 2 months. In order to determine growth performance, fish were weighed on weekly basis. Physico-chemical parameters of water i.e. dissolved oxygen, temperature and pH were monitored throughout the experimental session. Supplementation of myo-inositol significantly ($p<0.05$) improved the growth performance in terms of final weight, absolute weight gain, weight gain % and specific growth rate. The optimum myo-inositol requirement of *L. rohita* determined on the basis of weight gain% was 568 mg/kg of dry diet. Gills antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (POX) activities were linearly increased while thiobarbituric acid reactive substances (TBARS) contents were linearly decreased by increasing the myo-inositol supplementation level. Dietary myo-inositol supplementation significantly ($p<0.05$) increased the gills polyunsaturated fatty acids i.e. arachidonic acid, eicosapentaenoic acid, linoleic acid, n-6 and ARA/EPA ratio. However, supplementation of myo-inositol decreased the saturated fatty acids percentages in gills of fingerlings. In conclusion, dietary myo-inositol supplementation improved the growth performance, TBARS, antioxidant enzyme activities and polyunsaturated fatty acid percentages in gills of *Labeo rohita* fingerlings optimally at dietary dose of 568 mg/kg.

**CLONING, RELATIVE EXPRESSION AND MATING EFFECTS OF PBANR GENE IN
AGROTIS IPSILON (HUFNAGEL) (LEPIDOPTERA: NOCTUIDAE)
ADULTS BY USING qPCR**

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The black cutworm, *Agrotis ipsilon* is one of the destructive insect pest of most of the field crops of the world. Pheromone Biosynthesis-Activating Neuropeptide Receptor (PBANR) is a neuropeptide receptor that regulates and discharges the sex pheromone production in a variety of Lepidoptera species. One of the G Protein Coupled Receptors (GPCRs) plays a very important role to regulate sex pheromone biosynthesis in ligand to PBAN. Mating disrupts significant and permanent loss of sex pheromone production in adult Lepidoptera pests. The RACE-PCR was used to clone the full-length 3539bp nucleotide of pheromone biosynthesis activating neuropeptide receptor from the female pheromone gland of the adult black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) and named as (Aips-PBANR). The multiple protein sequence alignments revealed that proteins codified by *Agrotis ipsilon* PBANR gene shared high identity with homologs from other Lepidoptera organisms. The RT-PCR screening amplified preferably Aips-PBANR from pheromone gland of female and Male Aedeagus of male tissues. The real-time quantitative PCR (qRT-PCR) was used for investigating the expression profile of Aips-PBANR gene in different tissues, days and times. The qRT-PCR for tissue distribution resulted in Aips-PBANR expression maximum in pheromone gland; however a little was expressed in male aedeagus tissue. Further, the Aips-PBANR gene expression was found significantly highest at the 4th day after emergence, and 2:00 am in its scotophase period. In addition, qRT-PCR revealed that mating significantly inhibited Aips-PBANR expressions and suppressed pheromone production. The study concluded that PBANR is involved in multi-functional roles in cell activity, including sex pheromone reduction. These results provide solid framework to carry on essential functional studies of PBANR gene in *A. ipsilon* and other insect pests as pest management tool such as RNAi technique and other diversified functions of this diverse neuropeptide receptor. This work was supported by the China National Basic Research Program (2012CB114104) and the National Natural Science Foundation of China (31171858, 31321004 and 31471778), and Beijing Nova Programme (Z1511000003150118), China.

**EFFECTS OF CHROMIUM EXPOSURE ON ANTIOXIDANT ENZYME ACTIVITY IN
LABEO ROHITA AND CIRRHINA MRIGALA**

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Untreated run off water from agricultural land and industrial effluents contain toxic pollutants, including heavy metals, which can accumulate in the aquatic organisms, especially the

fish and cause oxidative stress. 120-day old fish fingerlings of *Labeo rohita* and *Cirrhina mrigala*, with three replications for each treatment, were exposed, separately, to 2/3rd, 1/4th and 1/5th of their respective 96-hr LC₅₀ values of chromium in glass aquaria for 30 days at controlled laboratory conditions. After 30-day exposure, metal stressed fish species were sacrificed and their organs (liver, gills, kidney, muscles and brain) separated for peroxidase enzyme assay. Chromium exposure induced significant variations in the activity of peroxidase in the selected organs of two fish species, *L. rohita* and *C. mrigala* as compared to control. In both fish species, peroxidase activity increased significantly after 30-day exposure due to enhanced production of reactive oxygen species by chromium while among organs, liver showed maximum peroxidase activity followed by gills, kidney, brain and muscles. Among treatments, fish species exposed to 2/3rd of 96-hr LC₅₀ of chromium exhibited significantly higher activity while among the fish species, *L. rohita* was more sensitivity as compared to *C. mrigala* as the variations in peroxidase activity were more pronounced in *L. rohita*.

DETERMINATION OF POST MORTEM INTERVAL VIA INSECTS

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In recent advanced era investigating legal cases relating to both humans and wildlife can be done with great accuracy with the help of forensic entomology. Forensic entomologist uses insects fauna colonized on carrion to determine elapsed time since death, cause of death and place of death. In the current study insect fauna on rabbit carcass at Mingora Swat Khyber pukhtunkhwa Pakistan during the month of June 2015. Five stages of decomposition namely the fresh, bloat, active decay, advanced decay and dry were observed. A total of 16 species were collected and identified. These species were representatives of the order Hymenoptera, Diptera and coleoptera. Among the order Hymenoptera two species of ants were collected. Similarly among the order diptera 8 species were collected and identified as member of family Muscidae, Calliphoridae, Sarcophagidae and piophilidae. 1 specie the *Musca domestica* represented the family Muscidae, 5 species namely *Lucilia sericata*, *Chrysomya megacephala*, *Chrysomya rufifacies*, *Chrysomya albiceps*, *Calliphora vomitoria* represented the family Calliphoridae, 1 specie *Sarcophaga haemorrhoidalis* represented the family sarcophagidae and 1 specie *Piophilidae casei* represented the family piophilidae. In the same manner among the order coleoptera 6 species were collected and identified. 2 species namely Clown beetle *Hister* specie and *Saprinus splendens* represented the family Histeridae, *Necrobia rufipes* represented the family cleridae, *Dermestes maculatus* represented the family Dermestidae, *Dactylosternum abdominale* represented the family Hydrophilidae, *Onthophagus expansicornis* represented the family Scarabidae. Ants, *M. domestica* and *L. sericata* visited the carcass during fresh stage, followed by *C. megacephala*, *C. rufifacies*, *C. albiceps*, *C. vomitoria*, *S. haemorrhoidalis*, Clown beetle and *Saprinus splendens* visited during the bloat stage. Besides this *N. rufipes*, *D. maculatus*, *D. abdominale* and *O. expansicornis* visited during active decay stage. Through their pattern of succession the PMI was estimated from 4.5-165 hours. The results will be definitely helpful in solving both the cases of human and wildlife in district Swat and acts as strong and convincing evidence.

GREEN SYNTHESIZED NANOPARTICLES USING *BERGENIA CILIATA* RHIZOME EXTRACT AND THEIR ANTIBACTERIAL AND CYTOTOXICITY EFFECT

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Many health hazardous diseases are cause by clinical pathogens. To overcome the effect of diseases and negative impact of chemically and physically synthesized nanoparticles. It is a serious concern to develop the new antimicrobial agent from nature and inorganic source which is environmentally friendly, less costly and more effective for the development of next generation drugs. So for this purpose *Bergenia ciliata* rhizome extract are used for the green synthesis of silver nanoparticle. *Bergenia ciliata* and silver nitrate both have medicinal properties. Synthesized silver nanoparticles are confirmed by UV-vis spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning electron microscope (SEM). These nanoparticles used to evaluate the antibacterial, antibiofilm, viability of cells, brine shrimp lethality and *in vitro* cytotoxicity assays. Spherical shaped synthesized nanoparticles were confirmed at 400 nm using SEM and UV-vis spectrophotometer. Greatest zone of inhibition (6.0±0.0 mm to 8.3±0.57 mm) was recorded against all tested pathogens compared with the *B. ciliata* aqueous extract. Antibiofilm analysis and MTT assay supported the results of antibacterial activity. It has been conclude that nanoparticles have bactericidal effect by degrading its DNA.

SILVER NANOPARTICLE SYNTHESIS USING *ARTEMISIA VULGARIS* LEAF EXTRACT AND THEIR BIOLOGICAL ACTIVITIES

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Infectious diseases are caused by etiological agents. Nanotechnology have used to minimize the effect of clinical pathogen which have the resistance against the antibiotics. The focus is on effective and efficient synthesis of silver nanoparticles while exploring their various prospective application. In current research synthesis, characterization and biological activities are screened. The characterization of silver nanoparticles was carried out using Fourier transform infrared spectroscopy (FT-IR), UV-Vis spectrophotometry and scanning electron microscopy (SEM). FT-IR indicated the involvement of the functional group in the preparation of silver nanoparticles. UV-Vis spectra shown absorption peak at 400 nm. SEM indicated the spherical shape of silver nanoparticles. Results reveal the antibacterial effect of green synthesized nanoparticles against clinical bacterial pathogens. Biofilm reduction and cell viability assay also

supported the antibacterial effect. Cytotoxicity of silver nanoparticles were recorded as 100% at 200 ug/ml through brine shrimp assay. Protein kinase inhibition zones recorded for the silver nanoparticles (16 mm bald) compare to *A. vulgaris* extract (11 mm bald), and surfactin established a 22 mm bald growth inhibition zone. It has been concluded that green synthesized silver nanoparticles are more effective against infectious pathogens and could be used as potential source of therapeutic drug.

**STUDY OF EXTRACELLULAR ENZYMES INVOLVED IN BIODEGRADATION OF
VAT DYES BY ASPERGILLUS FLAVUS**

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Current study focuses on biodegradation of disperse VAT dyes and study of lignolytic enzymes involved in biodegradation. Microorganisms (fungi) have great potential of biodegradation of dyestuffs due to their efficient nonspecific lignolytic enzyme system. In current study *Aspergillus flavus*, fungus isolated from *Ficus carica*, was employed. The targeted VAT dyes were VAT Violet 15, VAT Red 10, Indigo Dye, VAT Black 25, which are widely used in textile industries. Decolorization process was optimized by using Response Surface Methodology strategy. Initially decolorization was observed 92% VAT Violet 15, 88% VAT Red 10, 82% Indigo Dye, 72% VAT Black 25 ON DAY 5. There was 4%, 7%, 16% and 17% respectively increased in decolorization observed by the addition of readily available carbon and nitrogen sources. The maximum activities observed for lignolytic enzymes were 1892.76 U/ml for Lip, 1438.45 U/ml for MnP and 834.50 U/ml for laccase. Kinetic studies of lignolytic enzymes shows their efficiencies and specificities towards their substrates. The values of Km were found to be 0.751mM for Lip, 0.700 mM for MnP and 0.571 mM for laccase and that of Vmax were 1250 µM/ml/min for Lip, 1000 µM/ml/min for MnP and 1428.57 µM/ml/min for laccase. The optimum temperature was around 32.5 0C and optimum pH was around 6. It can be concluded from current study that *Aspergillus flavus* is a potential microorganisms for the treatment of industrial effluent. It can also be used for the production of active lignolytic enzymes. Further studies should be conducted to explore its potential against industrial effluent.

**CONSTRUCTION OF CELLULOLYTIC AND SULPHATE-REDUCING BACTERIAL
CONSORTIUM FOR ENHANCING EFFICIENCY OF CELLULOSE-LINKED
BIOREMEDIATION PROCESSES**

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Metallic and non-metallic pollutants originating from omnifarious industries are not treated before their final discharge into the environment. Consequently, environment is being degraded

very rapidly and posing serious threats to all forms of life. For remediation of the said pollutants, a number of physicochemical treatment methods have been practiced but couldn't found suitable due to environmentally non-compatible natures and generation of secondary pollutants. The present study was, therefore, designed to treat artificially prepared sulphate-rich wastewater jointly with the help of cellulolytic and sulphate-reducing bacterial species while using a variety of agro-industrial wastes as cost-effective growth substrates. In order to achieve the goal, the two bacterial species were mixed in different proportions to achieve significant results of sulphate reduction. Statistical analysis revealed that rice straw appeared as the most efficient carbon source among all the agricultural wastes because it reduced about 96 % of the total added sulphate in a 60-day trial of anaerobic incubation. Among all the industrial wastes, animal manure appeared as the most efficient carbon source, it could reduce 93 % of sulphate. Mixture of industrial and agricultural waste reduced about 90 % of the sulphate. Our findings of the present will be helpful in developing an economical and environmental friendly bioremedial technique for the treatment of metallic and non-metallic wastes simultaneously which ultimately convert the industrial wastewaters into harmless and suitable discharge to aquatic environment.

PURIFICATION AND CHARACTERIZATION OF URICASE ENZYME ISOLATED FROM *BACILLUS* SPP.

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Bacillus genus comprises variety of species that are diverse, commercially useful and widely distributed in nature. They produce a large number of enzymes that plays an important role in the medical, pharmaceutical, textile, leather, paper and food industry. The common example is uricase or urate oxidase enzyme that plays a key role in medical field as a therapeutic drug. This enzyme is absent in higher primates and can be produced from *Bacillus* spp. In this study, bacteria isolated and characterized from soil of 5 different locations in Lahore. A broth was prepared for the uricase enzyme production with different chemicals like KH₂PO₄ (0.2%), MgSO₄·7H₂O (0.1%), K₂HPO₄ (0.2%), NaCl (0.01%), CaCl₂ (0.01%) and uric acid (0.5%). *Bacillus* spp. produces extracellular enzyme and enzyme was assayed from extracellular medium (17.87 μmol/mL/min). Nutritional and physical parameters were optimized for maximum production of uricase. Best results were obtained when 0.5% uric acid used as an inducer, sucrose as a carbon source, peptone as a nitrogen source at pH 8 on 37°C for 36 hours. Ammonium sulphate precipitation was used for partial purification of uricase enzyme resulted in 4.67 fold purification with enhancement of specific activity of 301.5 μmol/mL/min. The molecular weight of uricase is 35 kDa determined by SDS poly-acrylamide gel electrophoresis. Uricase showed maximum activity at 37°C and pH 8. Kinetic characterization of uricase revealed uric acid as the highly specific substrate for the enzyme with Km value of 0.052 mg/mL and Vmax of purified uricase was 27.7 μmol/mL/min.

A STATISTICAL APPROACH TO STUDY ENHANCED THERMOSTABLE PROTEASE PRODUCTION FROM THERMOPHILIC *BACILLUS SUBTILIS* BSP STRAIN

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This research was aimed to obtain enhanced yield of extracellular thermostable protease from a thermophilic *Bacillus subtilis* BSP strain. Enzyme production conditions were optimized by using conventional one factor at a time study approach, fermenter level optimization and statistical response surface methodology. Conventional one factor at a time study approach enhanced (180 U/mL) protease yield at pH 8.0, 60°C and 1% casein concentration within 48 h. Optimization of physical factors (aeration and pH) in bench-scale bioreactor further enhanced enzyme production up to 248 U/mL at 0.8 vvm aeration and 150 rpm agitation within 48 h incubation. The combined effect of conventionally optimized variables (pH, time of incubation, casein concentration and inoculum density) by response surface methodology, yielded 304 U/mL of enzyme with 2% (w/v) casein, 1% (w/v) inoculum size, at 60°C and pH 8.0 within 72 h. This is the first report of a thermostable protease that retained its activity in the presence of high concentrations of surfactant (10% SDS) and oxidant (10% H₂O₂), a necessary trait for future applications in the alkaline detergent industry.

CONCOMITANT TREATMENT OF TANNERY AND PAPER MILL EFFLUENTS BY HIGHLY METAL-RESISTANT DISSIMILATORY SULPHATE-REDUCING BACTERIA

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A study was designed to treat tannery and paper mill effluents simultaneously using highly metal-resistant dissimilatory sulphate-reducing bacteria (DSRB) as remedial agents. In this study, the remedial efficiency of the bacteria appeared negligible when tannery and paper mill effluents were used solely without any growth substrate, while the efficiency of sulphate reduction and metal removal, appeared maximum when sodium lactate was used as growth substrate in another set of experiments. In this set of experiments, almost 100 % precipitation of the chromium (at 700 ppm) occurred in the first seven days of anaerobic incubation. Sulphate was also totally removed in the first seven days of incubation. pH of the media remained neutral to slightly acidic in range. The

findings of this study will be helpful in developing economical and environmental friendly bioremedial processes.

APPLICATION OF FRUIT WASTES AS COST-EFFECTIVE CARBON SOURCES FOR BIOLOGICAL SULPHATE REDUCTION

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We conducted a study dealing with the economical application of sulphate-reducing bacteria (SRB) for the treatment of sulphate-rich wastewaters. In the study, four types of frequently available fruit wastes were employed as carbon sources for economical propagation of the SRB and consequent reduction of sulphate from artificially prepared sulphate-rich wastewaters. The bacterial species, employed in this study, were isolated from a leading wastewater channel in Pakistan and characterized phenotypically as well as genotypically. The bacterial identities were proved after BLAST analysis and construction of phylogenetic tree. Among all of the SRB species employed for sulphate reduction, *Desulfovibrio fructosovorans*-HAQ2 was found as the leading sulphate reducer and reduced sulphate maximally to 29% ($0.51 \pm 0.02 \text{ gL}^{-1}$), 76% ($1.51 \pm 0.07 \text{ gL}^{-1}$), 41% ($0.83 \pm 0.02 \text{ gL}^{-1}$) and 63% ($1.25 \pm 0.02 \text{ gL}^{-1}$) using apple peelings, apple pomace, mango peelings and watermelon rind, respectively in a 60-day trial of anaerobic incubation. On average, watermelon rind appeared as the most appropriate carbon source for the SRB and all the SRB species reduced sulphate efficiently in the range of 40–63 % using this carbon source. Our findings of the present study highlight the productive utility of fruit wastes for developing cost-effective and environmental-friendly remedial strategies.

CHARACTERIZATION AND ANALYSIS OF BACTERIOCINS ISOLATED FROM LACTOBACILLUS SPP. USING YOGURT AS A PROBIOTIC FOOD CARRIER

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Antibiotics have been used in preventing and treating human and animal diseases for decades. It is better to use bacteriocins in place of antibiotics because bacteriocins are not toxic to consumers as well as there is no information on accumulation of bacteriocins in tissues. Lactic acid bacteria (LAB) are used as probiotics because they are the part of normal microbial flora of human and animal's intestine. LABs are anaerobic but aero-tolerant Gram positive rods or cocci which have capacity to ferment sugars for acid and energy production. During this study 8 probiotic LAB strains were isolated from various yogurt samples collected from Lahore region out of which 3 were selected (AnS1, AnS2 and AnH). They were grown under both aerobic and anaerobic conditions. Bacteriocins were isolated from strains AnS1, AnS2 and AnH (anaerobic strains) showing antimicrobial action against indicator strains *E. coli*, *S. aureus* and *L. monocytogenes*.

Filtered cell free extract of each strain was autoclaved to check the stability of bacteriocins present at high temperature. Antimicrobial activity assay later indicated that these bacteriocins were heat-labile peptides. The extracellular bacteriocins were analyzed on 12% SDS-PAGE. The results revealed that mol. weights of secreted proteins were 65 and 55 KDa. Protein quantification was done using BSA method, showed that approximately 2 to 5 mg/ml of each bacteriocin was present. From the RP-HPLC it was concluded that bacteriocins present could be of Class III bacteriocin that are not heat stable. Further HPLC analysis showed the number of peptides present in our samples as each peak showed a single purified protein. There is Nisin Z or its variant is present in the AnS2 samples. 16S rDNA sequence analysis showed 95% similarity to genus *Lactobacillus*.

MAGNIFIED YIELD OF CELLULASE BY *BACILLUS AMYLOLIQUEFACIENS*-ASK11 UNDER HIGH CHROMIUM STRESS

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Chronic exposure of soil to metal pollutants makes it imperative to study agro-ecological responses of nutrient recycling microbiota of the soil under metal stress conditions. In this connection, we conducted a study to check the production of cellulase by a chromium-resistant cellulose degrading bacterial strain, isolated from a leather-tanning industrial waste-contaminated area. The isolate was identified as *Bacillus amyloliquefaciens*-ASK11 through 16S rDNA sequencing. The supplementation of galactose and peptone as carbon and nitrogen sources, respectively enhanced the production of cellulase significantly. The bacterium yielded the production of cellulase to 20.23 U mL⁻¹ in optimized media with 10 ppm of Cr(VI). The increments in cellulolytic activity were achieved by cultivating 10 % inocula of *B. amyloliquefaciens*-ASK11 at pH 7 (28 °C) with aeration at 120 rpm for 96 h. Inhibitory relation between increasing chromium concentrations and the cellulolytic activity could permit the isolate, *B. amyloliquefaciens*-ASK11 to express 1.33 U mL⁻¹ of cellulase at 500 ppm of Cr(VI). It is, therefore, suggested that chromium-resistant cellulolytic bacteria could be exploited for the rehabilitation and bioremediation of chromium-ruined agro-industrial soils with concomitant gearing of carbon cycle.

PRODUCTION AND PURIFICATION OF CELLULASE FROM *ASPERGILLUS NIGER* USING RICE POLISH AS GROWTH SUBSTRATE

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Cellulases collectively make a group of hydrolytic enzymes and are capable of degrading lignocellulosic materials. The cellulases have wide range of applications. The present study focuses

on factors relevant for improvement of enzymatic hydrolysis of rice polish by using *Aspergillus niger*. Different cultural conditions were examined to assess their effect in optimizing enzyme production. Alkaline pretreated (NH₂SO₄) rice polish (at 70% concentration) resulted in cellulolytic activity of 99 U mL⁻¹ min⁻¹. The optimum pH for cellulase production appeared between 4.0 and 4.8. At optimum temperature (35°C), the enzymatic activity appeared upto 98 U mL⁻¹ min⁻¹.

ISOLATION AND PRODUCTION OF ALKALINE PROTEASE ENZYME FOR DEHAIRING OF ANIMAL SKIN

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The isolated bacterium from natural sources forms spores under adverse conditions and was screened and confirmed as gram positive *Bacillus* spp. by various tests such as gram staining, spore staining, API 50 CHB and by Bergy's Manual of systematic Bacteriology. It was determined by growth curve that the growth of organism increases with the increase of incubation period and reached at its maximum at around 24 hours and at around 26 hours of culture the protease activity was found as maximum. *Bacillus* spp. produces extracellular protease in growth medium and was best grown at optimum pH and temperature 8.5 and 60°C respectively. The enzyme hydrolyses proteins like hydrolysis which suggested it an extracellular alkaline protease and showed de hairing from skin and hides. The potential for use of microbial enzymes in leather processing lies mainly in areas in which pollution-causing chemicals are being used.

PREDICTING THE POTENTIAL HYBRIDIZATION BETWEEN *LABEO ROHITA* AND *CATLA CATLA* USING MICROSATELLITE MARKERS

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Major carps are commercially important aquaculture freshwater fish species of Pakistan. One of the concerns is increased inadvertent hybridization between major carps hatchery reared stocks. The determination of pure species or hybrid status of *Labeo rohita* and *Catla catla* is essential for the appropriate management of their breeding on fish farms. Domestic stocks of these species are also released in wild environment for fisheries rehabilitation purposes where these species may pose endanger to the wild populations through gene pool contamination. The use of microsatellites and new Bayesian models based statistical methods, have dramatically improved the assessment of admixture analyses and individual assignment testing. In present study, a set of eight heterologous microsatellite markers were used to assess the extent of potential hybridization in stocks of *L. rohita* and *C. catla* collected from Public fish seed Hatchery, Faisalabad. The methods, STRUCTURE assignation and NEWHYBRIDS status determination statistical analyses were used to determine the status of pure and hybrid individuals. Bayesian model-based clustering analysis revealed the presence of two taxa crossponding to each analyzed species. Individual admixture coefficients showed that 18% of hatchery collected specimens of putative *L. rohita* and *C. catla*

were hybrids and all the detected hybrids were categorized as F1 hybrids by NEWHYBRIDS approach. The Bayesian model statistical analyses showed a high efficiency in discriminating pure species and hybrid specimens. The DNA markers and admixture analyses can be fruitfully used to identify genetically pure or hybrid populations in captivity as well as in nature. The current genetic monitoring and the dissemination of practical data should support the conservation actions such as to enforce strict controls of the genetic status of captive stocks used for restocking in wild environment.

A REVIEW OF BIOGAS PRODUCTION THROUGH DIFFERENT SOURCES AND ROLE OF ITS RESIDUES IN INDUSTRY AND AGRICULTURE.

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Fossil fuels like coal and petroleum are depleting in world, due to increasing a number of population day by day, so alternative sources of energy is the requirement of the present day for overcome these energy crises. One amongst the major challenging source of energy is biogas being produced from biomass. The aim of this study is to give a review of different sources of biogas production. For example sewage waste, fruits and vegetable, slaughter house waste, animal waste, flowers waste etc are commonly used in biogas production. This study is conducted to aware people about the magnificent benefits of biogas residue as fertilizer in the field of agriculture and energy production in industrial field. Distinctly in the rural areas where a massive amount of natural waste is accessible without proper usage, by utilizing these wastes material in manageable way we resolve the energy problem in the foam of production of biogas.

MYCOSYNTHESIS OF SILVER NANOPARTICLES BY *ASPERGILLUS FUMIGATUS* BTCC10 AND OPTIMIZATION OF PROCESS VARIABLES USING STATISTICAL EXPERIMENTAL DESIGN

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Silver nanoparticles can be synthesized by using various chemical, physical and biological methods however the biological method is the most efficient, inexpensive, nontoxic and environmental friendly. Silver nanoparticles were produced and optimized by using *Aspergillus fumigatus* (BTCC10) with the help of Response Surface Methodology (RSM) and Central Composite Design (CCD). The variables selected for optimization were substrate concentration (mM), concentration of NaCl (%) and weight of biomass (wet weight) (g). The experimental design had 20 runs and the smallest particle size was obtained with 2 mM concentration of silver nitrate, biomass wet weight 5.977g and concentration of NaCl at 15%. Silver nanoparticles were characterized by Dynamic Light Scattering (DLS) Zeta sizer which showed size of 33.23 nm (100% monodispersion) and zeta potential measurements exhibited a negative zeta potential peak (-9.91mV) of the optimized silver nanoparticles. Rest of the characterization was done by UV-Vis spectrophotometer (410 nm) and ATR- Fourier Transform Infrared spectroscopy (FT-IR) (O-H,

C=O) and Atomic Force Microscopy (AFM). About 78.7 % of reduction in size was observed during the study. The results reflected that the RSM model is significant, with F-value of 3.91 and probability P value of < 0.0224. As coefficient of variation is indicative of degree of precision therefore, CV (37.53 %) approved the reliability of the model. However, $R^2 = 0.7786$ also added to the accuracy of the model. Then presence of nitrates was studied by Nitrate Reductase Assay (enzyme assay) and highest concentration of 489.5 ppm was obtained, followed by testing antimicrobial activity against different Multiple Drug Resistant (MDR) strains which showed maximum zone of inhibition (16.5mm) at 7 µg/ml with *Klebsiella pneumoniae*. Also, silver nanoparticles and their synergistic effect with the antibiotic Streptomycin was also checked which showed a maximum zone of inhibition (15mm) with *Klebsiella pneumoniae*.

ISOLATION AND SCREENING OF CELLULYTIC BACTERIA FROM COMPOST

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Cellulose is an important constituent of agriculture wastes. Composting is the process of degradation of waste materials. During the present work isolation of novel microbial species involved in breakdown of cellulose in composting process was focused. Bacteria were isolated from different composting samples. CMC media was used for screening of bacteria. The highest potential to degrade cellulose was recorded for four *Bacillus* strains. Molecular characterization of potential cellulolytic bacteria by 16S rRNA was performed. *Bacillus* sp. were found as most prevalent cellulolytic bacteria in composting process. Hence these bacteria can be used as an inoculum for degradation of agricultural wastes having cellulose.

CHARACTERIZATION OF AZO DYES REDUCING BACTERIAL STRAINS ISOLATED FROM INDUSTRIAL WASTEWATER

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A developing country like Pakistan requires the establishment of new industries in order to develop its economy. Synthetic dyes especially azo dyes which are widely used in many industries such as textiles, cosmetics, paper, leather and pharmaceutical, are the major constituents of the wastewater discharged from such industries. Due to its non-degradability and biomagnifications properties, it persists in the environment and imposes significant effects on human and animal health. However, microorganisms including bacteria are responsible for their degradation under anaerobic and aerobic conditions which is eco-friendly and cost-competitive alternative to chemical decomposition process. The present study aimed at isolation and characterization of azo dye resistant bacteria as well as to analyze the degraded products of azo dye produced by bacteria.

Wastewater samples were collected from Kot Lakhpat, an industrial estate, Lahore. Azo dye resistant aerobic bacteria were isolated and characterized by biochemical and 16S rRNA ribotyping. The dye degradation conditions i.e., temperature, pH, incubation condition, carbon and nitrogen sources, inoculum percentage and dye concentration were optimized on lab scale. For further confirmation of degraded products, FTIR, HPLC and TLC were performed. These bacterial strains may find potential applications in waste treatment strategies to decontaminate wastewater containing azo dyes.

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

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

ISOLATION, OPTIMIZATION AND APPLICATIONS OF LYSINE BY MICROBIAL FERMENTATION USING DIFFERENT BACTERIAL STRAINS

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Out of the nine essential amino acids, lysine is the second most important amino acid used as animal feed, food and pharmaceutical industries. It can be produced in different ways, among them fermentation is the most economical and practical means of producing lysine. In this method low temperature, low pressure and low-cost carbon sources are used and a biological form of lysine (L-lysine) is produced. The present work was planned to maximize yields of free lysine produced in a culture broth by different bacterial strains recovered from different soil and water samples. In

order to isolate the bacterial strains, tri-sector cultivation was performed in Plate Count Agar media and was then identified by studying the morphological characteristics. Best performed bacterial colonies were identified using Polymerase Chain Reactions (PCR). For qualitative assay, ascending Thin Layer Chromatography (TLC) was employed for the detection of lysine in the culture broth. Substrate, temperature, pH, incubation period and metal ions concentration are some of the optimizing parameters that were performed respectively. Spectrophotometric analysis was accomplished in order to assess quantitatively. High Performance Liquid Chromatography (HPLC) was subjected for final estimation of lysine in respective medium. Crude form of lysine obtained was then used in the chick feed and observed the growth change.

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

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

CULTURING OF THERMOPHILIC ANOXYBACILLUS BEPPUENSIS IN AGRO-DAIRY WASTES

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A thermophilic bacterium was isolated from hot spring located near the city Chakwal in Pakistan. The cells of strain were rod-shaped, stained Gram positive and are endospores formers. The isolates were able to utilize sugars like fructose, maltose, mannose, xylose, saccharose, sorbitol, mannitol, Arabinose and polysaccharides like gelatin and starch. The isolates grew over a wide range of temperatures (40-70°C) and pH (4-9). The isolates exhibited growth with variety of nitrogen sources. Optimum temperature for growth of both *A. beppuensis* JF84 was observed at 60°C. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain belonged to the *Anoxybacillus beppuensis* with 99% similarity under Accession number KF254912 (JF82) and DNA G + C content was 56.596. The isolate was grown on agro-dairy wastes to attain their cheap production by utilizing the wastes nutrients. Protein production was determined using 1% (w/v) of various agro-dairy wastes in production medium both with and without nutrients (g/100mL):

K₂HPO₄ (0.1), (NH₄)₂SO₄ (0.1), NaCl (0.1), MgSO₄ (0.1) at pH 7.0 after incubation of 48 h at suitable temperatures. It yielded enough thermostable protein suggesting their potential in production of various enzymes and proteins in unconventional and economical substrates suitable for various industrial uses.

BIOGAS PRODUCTION FROM DIFFERENT SOURCES IN SAARC COUNTRIES

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Energy crisis is the dilemma of modernization. SAARC countries being a one of the fastest growing region in the world, bearing 23% of world population is severely suffering from energy shortfall. The region has endowed with a variety of energy resources owing a greater potential of energy sharing for regional prosperity and socio economic development. India and Pakistan are the main countries of this region having a potential to fulfill 56% of the energy demand of the region. In this region, most of the people rely on fossil fuels by traditional means. Such practices are the source of significant environmental, social, economic and health issues. In addition to this, there exists a wide variation in commercial energy endowments and commercial energy demand. One of the main reasons behind all these is the lack of regional cooperation in producing power from available resources. To achieve sustainable development in this area, it is vital to gain access to affordable and clean energy. In this particular context, upgrading existing biomass resources (i. e., animal manure, crop residues, sewage waste and solid wastes) to cleaner and more efficient energy carriers (such as biogas from anaerobic digestion) has unique potential to provide clean and reliable energy, while concurrently preserving the local and global environment. The intent of this paper seeks to highlight a comparative and comprehensive review of SAARC renewable energy sector (biogas) and possible sharing opportunities. Biogas production have been discussed and compared in context of SAARC energy requirements. SAARC relations and energy sharing opportunities have also been elaborated in context of regional peace and security situation. Exploitation of the SAARC energy potential and energy sharing opportunities might chip to global peace and prosperity.

IN SILICO DVL3 PATHWAY ANALYSIS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA TO IDENTIFY DRUG TARGETS

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Disheveled (DVL) proteins are the key regulators of Wnt signaling cascade components and their over expression is strongly associated with cancer progression, being involved in cell proliferation regulation in humans. The present work analyzed and characterized the contribution of Disheveled segment polarity protein 3 (DVL3) mediated network in Head and neck squamous cell

carcinoma (HNSCC). STRING database was used to retrieve the network of DVL3 as a query submitted and Web Gestalt toolkit was used for enrichment analysis of network that provided disease sensitive proteins. Hub proteins were extracted from Hubba server and finally cBioPortal a genomic cancer platform was used for further mutational analysis. Several protein's over/under expression was clearly scrutinized that are involved in cell proliferation and apoptotic activities such as DVL3, FGF3, PIK3CA, CDH19, FAT1, PRKCI, DLG1, CTTN, EPHB3, GNB4, PSMD2, FGF4, TP53, PAK2, MYC and NOTCH1. These proteins are declared as confirmed drug targets in HNSCC. These findings will pave a repository for rational, empirical and experimental future investigation of therapeutic markers of DVL3 network in HNSCC.

THE *IN SILICO* STUDY OF POSTTRANSLATIONAL MODIFICATIONS OF HISTONE H2A.X IN DNA REPAIR

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Damage of DNA such as breakage of the double helix or double-strand breaks (DSBs) can be caused by different external and internal factor, which can have deleterious consequences like cell death. DNA damage and repair include chromatin remodelling and histone modifications. The family of histone proteins consist of four core (H2A, H2B, H3 and H4) and one linker (H1) protein of basic nature, which is organized with eukaryotic DNA into a compact chromatin structure. Amongst the core histone proteins, H2A has the largest number of variants mostly differing in their C-terminus. One of the most common H2A variant is H2A.X mostly found near DNA-damage sites, where it is H2A.X phosphorylation on Ser139. Phosphorylation is an important conserved modification playing an important role in early response to induction of DNA damage in multiple eukaryotic organisms, and has been suggested as a marker for DNA damage and repair. Phosphorylation of H2A.X on Ser139 is catalyzed by the kinase ATM, and is the first step in recruiting and localizing DNA repair proteins. Once DNA is repaired H2A.X becomes dephosphorylated by protein phosphatase 2A. Another equally important single moiety modification is *O*-GlcNAc modification occurring on Ser and Thr residues in a protein. Phosphorylation and *O*-GlcNAc modification can in some instances work in an inversely manner, when these modifications take place on the same and/or neighboring Ser/Thr residues. These sites are called Yin Yang sites. In this work phosphorylation, glycosylation and Yin Yang sites were predicted using bioinformatics tools such as Netphos 3.1 and YinOYang 1.2. The predicted Yin Yang sites were found to be Ser1, 16, 19, 121, 130, 139 and Thr101, 120, 136. Ser139 is a predicted Yin Yang sites (Ser139 can be phosphorylated at one time and *O*-GlcNAc modified at another time) and a fully conserved residue. The current study suggests that DNA damage and repair is regulated by H2A.X phosphorylation and *O*-GlcNAc modification. When DNA undergo single or double strand breakage H2A.X is recruited to the site, where it becomes phosphorylated on Ser139; whereas when DNA has been repaired H2A.X becomes dephosphorylated and consequently *O*-GlcNAc modified. These findings show the importance of post-translational modifications in DNA damage and repair, and can be useful in providing insight into higher order chromatin structures.

2. CELL BIOLOGY, GENETICS

BONE MARROW: A PROMISING AND HIGHLY PLASTIC SOURCE FOR STEM-CELL THERAPY OF MULTIPLE DISEASES AND DISORDERS

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Bone Marrow, being a vat of multiple cell types in extensive and immense quantity, is a source of cure to multiple disease and disorders. After recent research, it is found to be one of the most promising sources of stem cells capable of dealing almost all organs of the body either lost their anatomical continuity or the physiological. In this review, all the maladies which BMT could cure are addressed. From auto-immune disease to acquired immuno-deficiencies and from simple wound healing to myocardial repair, bone marrow is The Holy Grail, containing cure to all.

OSTEOGENIC POTENTIAL OF HERB *CISSUS QUADRANGULARIS*: EFFECT OF HEXANE FRACTION (CQ-HEX) ON GROWTH PARAMETERS AND PROLIFERATION OF MOUSE PRE-OSTEOBLAST CELL LINE MC3T3-E1 (Sub Clone 4) AND HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS hAMSC

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Plants (Herbs) are mostly used as traditional medicines and many such medicinal plants are reported in Ayurveda, Chinese and Unani literature. *Cissus quadrangularis* (Common name: Haddjod or Bone Settler) is one of such medicinal herbs which has been reported in Ayurveda literature for its various pharmacological properties especially Healing Bone fractures. It has also been reported for its anti-inflammatory, anti-analgesic, anthelmintic, anti-diabetic, anti-obesity, anti-ulcer, anti-hemorrhoid etc. properties. The present study is part of a project which explores the osteogenic potential of *Cissus quadrangularis* (CQ). Crude Ethanolic extract of CQ was prepared and further fractionated into four purified fractions i.e. n-Hexane (CQ-Hex), Dichloromethane (CQ-DCM), n-Butanol (CQ-But) and Ethyl Acetate (CQ-EA). In this part of the study, the effect of Hexane fraction of CQ was studied on the proliferation and growth parameters of MC3T3-E1 mouse pre-osteoblast cell line and human adipose-derived Mesenchymal Stem Cells (hAMSC). Nine concentrations (200µg/ml – 0.0001µg/ml or 0.1 ng/ml) of CQ-Hex were tested and our preliminary results indicate that four concentrations of CQ-Hex were non-toxic for the cells. The results are supported by growth curve analysis and various assays to analyze cytotoxicity and cell proliferation. Further investigation is going on to study the effect of CQ-Hex on mineralization potential of MC3T3-E1 cell line as well as mineralization of hAMSCs, up to molecular level.

RIBOSOMES MAKE SWEEPING ARRESTS

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Regulation of gene expression is a vital process for all organisms. One regulation mechanism uses arrest peptides, sequences that pause translation in cis, stalling the ribosome that just synthesized them. The arrested ribosomal complex changes the conformation or localization of its mRNA, modulating its translation. Most arrest peptides are active only in the presence of specific environmental cues and modulate the cell's response to those cues, such as the upregulation of antibiotic-resistance genes upon exposure to antibiotics. Current work suggests that small changes in the nascent peptide dictate the ability of ribosomes to respond to this and other small molecules.

OSTEOGENIC POTENTIAL OF HERB *CISSUS QUADRANGULARIS*: EFFECT OF DICHLOROMETHANE FRACTION (CQ-DCM) ON DIFFERENTIATION OF MOUSE PRE-OSTEOBLAST CELL LINE MC3T3-E1 (SUB CLONE 4)SHABANA MALIK, RABAIL HASSAN TOOR, FAIZA BATOOL AND
ABDUL RAUF SHAKOORI*School of Biological Sciences, University of the Punjab, Lahore*

Plants (Herbs) are mostly used as traditional medicines and many such medicinal plants are reported in Ayurveda, Chinese and Unani literature. *Cissus quadrangularis* (Common name: Haddjod or Bone Settler) is one of such medicinal herbs which has been reported in Ayurveda literature for its various pharmacological properties especially Healing Bone fractures. It has also been reported for its anti-inflammatory, anti-analgesic, anthelmintic, anti-diabetic, anti-obesity, anti-ulcer, anti-hemorrhoid etc. properties. The present study is part of a project which explores the osteogenic potential of *Cissus quadrangularis* (CQ). Crude Ethanolic extract of CQ was prepared and further fractionated into four purified fractions i.e. n-Hexane (CQ-Hex), Dichloromethane (CQ-DCM), n-Butanol (CQ-But) and Ethyl Acetate (CQ-EA). In this part of the study, the effect of Dichloromethane fraction of CQ (CQ-DCM) was studied on growth parameters and mineralization potential of MC3T3-E1 mouse pre-osteoblast cell line. The cytotoxic analysis was carried out by Neutral red assay and of nine concentrations (200 µg/ml – 0.0001 µg/ml or 0.1 ng/ml) of CQ-DCM tested, of which three concentrations were found to be non-toxic to the cells. These results were supported by growth curve analysis, metabolic activity assay, and proliferation assay. MC3T3-E1 cells were induced to differentiate into osteoblast cells in presence of osteogenic medium for 21 days. The non-toxic concentrations of CQ-DCM were tested against positive and negative control for their effect on mineralization of MC3T3-E1 cell line. Enhanced and early mineralization was observed in presence CQ-DCM as compared to positive control. The results are supported by VonKossa staining, Alizarin Red S staining and ALP staining of differentiated cells. The expression of bone specific marker proteins also studied. Further investigation is going on to fully understand how different fractions of CQ effect the proliferation and mineralization of MC3T3-E1 cells up to molecular level.

OSTEOGENIC POTENTIAL OF HERB *CISSUS QUADRANGULARIS*: EFFECT OF ETHYL ACETATE AND N-BUTANOL FRACTION ON DIFFERENTIATION OF MOUSE PRE-OSTEOBLAST CELL LINE MC3T3-E1 (SUB CLONE 4)

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Plants (Herbs) are mostly used as traditional medicines and many such medicinal plants are reported in Ayurveda, Chinese and Unani literature. *Cissus quadrangularis* (Common name: Haddjod or Bone Settler) is one of such medicinal herbs which has been reported in Ayurveda literature for its various pharmacological properties especially Healing Bone fractures. It has also been reported for its anti-inflammatory, anti-analgesic, anthelmintic, anti-diabetic, anti-obesity, anti-ulcer, anti-hemorrhoid etc. properties. The present study is part of a project which explores the osteogenic potential of *Cissus quadrangularis* (CQ). Crude Ethanolic extract of CQ was prepared and further fractionated into four purified fractions i.e. n-Hexane (CQ-Hex), Dichloromethane (CQ-DCM), n-Butanol (CQ-But) and Ethyl Acetate (CQ-EA). In this study, the effect of two of purified fractions viz., Ethyl Acetate fraction of CQ (CQ-EA) and n-Butanol fraction of CQ (CQ-But), was studied on growth kinetics and mineralization potential of mouse pre-osteoblast cell line MC3T3-E1 (subclone 4). The results indicate that both CQ-EA and CQ-But fractions enhance mineralization of MC3T3-E1 cell line. In both cases, of six concentrations (200µg/ml – 0.01 µg/ml) tested, of which four concentrations were found non-toxic for the cells. The results were supported by cell viability/cytotoxic assay, and cell proliferation assay. The non-toxic concentrations of both CQ-EA and CQ-But were tested for their mineralization potential of MC3T3-E1 cells in presence of osteogenic medium for 21 days against positive and negative controls. Cells were fully mineralized (differentiated) by Day 14 of treatment in positive control and CQ (EA and But) treated wells. Both fractions of CQ (EA and But) enhanced the mineralization of MC3T3-E1 cell line as compared to the positive control. The results are supported by VonKossa staining, ALP staining and Alizarin Red staining of mineralized cultures as well as gene expression patterns of bone marker proteins by real-time PCR. Further study is going on to investigate the effect of purified fractions of CQ on lineage commitment of hMSCs to osteoblasts, and on the signaling pathways involved in the bone formation process.

GENOME-WIDE ASSOCIATION STUDY OF CAT MAMMARY TUMOR USING 63,000 SNP CHIP THROUGH PLINK DATA ANALYSIS TOOLSET

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Early disease diagnosis is important to ensure the animal welfare, so disease associated mutations may serve as one of the best tool to take timely decisions where the breeding process is involved. A genome wide case-control association study using the Illumina Infinium *Feline* 63K

DNA chip was used in 7 mammary tumors and 123 controls. Array-based genotyping data was analyzed by PLINK data analysis toolset. Different tests were performed to determine the allelic and haplotype association of the SNPs with mammary cancer. The sample showed zero Mendel error and deviation from the Hardy Weinberg Equilibrium. Allelic association test revealed 10 most associated SNPs with this tumor type shown in Manhattan plot. Similarly, LD plot depict that one of the SNP exist as haplotype within RFTN 2 gene. This protein is involved in the activation of immune cells. Another SNP on Chr.14 was found to be in linkage disequilibrium with C7orf64, APPBP2, PPMID and BCAS 3 genes, which are highly expressed in breast cancer and appearing as a haplotype. This GWAS study revealed that mammary tumor in Siamese cat is cumulative effect of number of SNPs located on different chromosomes which may be helpful to better understand the diagnosis and association genetics of this tumor type.

MOLECULAR CHARACTERIZATION OF POSTERIOR CORNEAL DYSTROPHIES FROM PUNJAB

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Visual perception is a main attribute of living beings which helps them to see the surrounding environment and deformities in vision lead to visual impairment. Corneal dystrophies are group of inherited corneal diseases causing changes in the different layers of cornea. Posterior corneal dystrophies are characterized by abnormalities of the corneal endothelial and descemet membrane. *COL8A2*, *SLC4A11*, *LOXHDI*, *VSXI*, *KCNJ13*, *ZEB1*, *TCF8* genes cause Posterior corneal dystrophies. Small aggregates of apparent vesicles bordered by a grey haze and geographic areas appear clinically at the level of descemet or excrescences on a thickened descemet membrane are clinically found. The purpose of this study was to comprehensively characterize Posterior corneal dystrophy from population of Punjab. Identification and registration of five families with PCD was carried out with the help of ophthalmologists from different hospitals of Punjab. DNA isolation was done from blood samples of these affected families and desired gene *SLC4A11* was amplified via PCR with three markers D20S181, D20S193 and D20S889. Genetic analysis of one family PKCD005 was carried out at molecular level and it was found that all the three markers are linked to the reported *SLC4A11* gene. Patients and their families were made aware about the treatment and prognosis of the disease.

STUDY OF GENES INVOLVED IN HEREDITARY MICROCEPHALY FROM AZAD JAMMU AND KASHMIR.

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Autosomal recessive primary microcephaly (MCPH) is a rare genetic disorder in which the patients show reduced head circumference and cognitive defects. To date, 17 MCPH genes have been identified in which eleven are reported in the last five years, showing the rapid progress in this field. Still, families phenotypically affected with microcephaly that do not show any mutations in these

known MCPH genes exist, demonstrating that some new genes may be involved in microcephaly. Here, we report the results of a genetic study on a cohort of 15 consanguineous families (46 affected individuals) with an MCPH phenotype from the Azad Jammu and Kashmir (AJK) territory in Northern Pakistan. All families were examined clinically on field trips with microcephaly detected in all affected individuals but no other obvious malformations. Applying whole exome sequencing, followed by Sanger sequencing, we identified mutations in the four known MCPH genes *ASPM* (MIM*605481), *CENPJ* (MIM*609279), *MCPHI* (MIM*607117), *STIL* (MIM*181590). We detected one novel homozygous frame shift mutation (c.5606_5607insC, p.H1870Tfs*26) in the *ASPM* gene predicted to result in a truncated protein in one family. Further 12 MCPH families carried previously reported homozygous mutations in the *ASPM* gene: (i) the frame shift mutation c.8200_8201delAA (p.N2734Lfs*16) in five families, (ii) the nonsense mutation c.2967G>A (p.W989*) in three families, (iii) the missense mutation c.9539A>C (p.Q3180P), the nonsense mutations c.9190C>T (p.R3064*), c.2938C>T (p.R980*) and the frame shift mutation c.7782_7783delGA (p.K2595Sfs*6) in one family each. Family MC6 carried a novel homozygous splice site mutation on chromosome position chr1:197125193A>G (GRCh38.p2 assembly, NC_000001.11) in addition to the missense mutation c.9539A>C (p.Q3180P). In one family the homozygous frame shift mutation c.17delC (p.S7Lfs*4) in the *CENPJ* gene was identified. Family MC4 carried a heterozygous missense mutation in the *MCPHI* (c.2422G>A; p.V808I) as well as in the *STIL* gene (c.1136C>T in transcript variants 1-4; c.995C>T in transcript variants 5 and 6; p.S379F in isoforms 1-3; p.S332F in isoforms 4 and 5. While homozygosity of these mutations is known to cause MCPH1 and MCPH7 respectively, it can only be speculated at this point that a combination of heterozygous mutations in two different MCPH genes can potentially result in an MCPH phenotype. Further functional analysis and analysis of other possible candidate genes will be necessary to identify the MCPH-causing mutation in this family.

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISM IN *CD28*(C/T-I3 + 17) AND *CD40* (C/T-1) GENES WITH THE GRAVES' DISEASE

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Graves' disease (GD) is an organ-specific, multifactorial, heterogeneous autoimmune disorder affecting the thyroid, eyes and skin and develops due to complex interaction of genetically susceptible genes and environmental factors. The aim of this case-control study was to find out the correlation between the single nucleotide polymorphisms (SNPs) in *CD28* [(C/T-I3 + 17) (rs3116496)] and *CD40* [(C/T-1) (rs1883832)] genes with GD, if any, by using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. A total of 80 GD patients were enrolled from Multan Institute of Nuclear Medicine and Radiotherapy (MINAR) in Multan, Pakistan and age matched 80 controls were also recruited from local population. Various risk factors were also correlated either with the genotype at each SNP or with various combinations of genotypes studied during present investigation. Our results indicated that both SNPs in both genes were not associated with the GD either individually or in any combined form. Risk factor analysis results revealed that gender ($P = 0.008$), marital status ($P < 0.000$), education ($P < 0.001$), smoking ($P < 0.000$), Tri-iodothyronine (T_3) ($P < 0.000$), thyroxine (T_4) ($P < 0.001$) and thyroid stimulating hormone (TSH) ($P < 0.000$) levels in blood were found associated with GD.

GENETIC BASIS OF ALOPECIA IN THREE CONSANGUINEOUS FAMILIES FROM SOUTHERN PUNJAB

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Atrichia with papular lesions (APL) is a rare autosomal recessive form of total alopecia, characterized by hair loss soon after birth and the development of papular lesions of keratin-filled cysts over extensive areas of the body. Two consanguineous families were enrolled from Basti Mochi Wala, Mouza Gulab Shah in Muzaffargarh District having multiple siblings suffering from alopecia. The aim of this study was to find out the genetic mutation(s) in hairless (HR) gene, if any, in the enrolled subjects. A questionnaire was filled for each subject on the sampling site in order to collect epidemiological data associated with the disease. Patients from both families exhibited congenital Atrichia with papular lesions (APL) including hair loss in the scalp, pubic and other body parts. Polymerase chain reaction (PCR) was used to amplify all the overlapping intron exon regions of HR gene followed by DNA sequencing. Analysis of the DNA sequence revealed a novel deletion mutation (429delC) in exon 2 of HR gene leading to frameshift that resulted in to premature termination codon in mRNA of HR gene resulting in a truncated protein with 166 amino acid residues. The mutation followed Mendelian pattern of inheritance as all the patients had homozygous mutation while parents were heterozygous and unaffected siblings, from both families, were either heterozygous for the reported mutations or they lack this particular mutation.

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN *XRCC1* (194) AND *XPD* (751) WITH AGE RELATED CATARACT

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Age-related cataract (ARC) is a multifactorial disease and different risk factors, including genetic and environmental, are responsible for the development of its various types. The aim of this study was to find out a correlation, if any, between age related cataract (ARC) and the single nucleotide polymorphisms (SNPs) in DNA repair genes *XRCC1* (X-ray repair cross-complementary-1) [Arg194Trp (rs1799782)] and *XPD* (Xerodermapigmentosa complementation group D) [Lys751Gln (rs13181)]. The genotype at these two SNPs was analyzed in 260 subjects (125 control and 135 patients) from Southern Punjab population (Pakistan) by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. Genotype at both analyzed codons was correlated either individually or in various combinations with the studied epidemiological factors known to be associated with ARC. Our results indicated that both SNPs Arg194Trp in *XRCC1* ($P = 0.967$) and Lys751Gln in *XPD* ($P = 0.995$) were not associated with ARC whether they were analyzed individually or in combined form ($P = 1$). Analysis of epidemiological factors revealed that age ($P < 0.001$), cast of subjects ($P = 0.001$), diabetes ($P < 0.001$), hypertension ($P = 0.001$), smoking habit ($P = 0.01$), drug abuse ($P < 0.05$), steroid use ($P = 0.001$) and body weight ($P < 0.001$) can influence the incidence of ARC in studied subjects. It is concluded that studied SNPs in *XRCC1* and *XPD* have no association with the incidence of age related cataract in the analyzed group of subjects.

GENETIC ANALYSIS OF NATURAL AND HATCHERY REARED POPULATIONS IN MAJOR CARP (*CIRRHINUS MRIGALA*)

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In Pakistan, polyculture of major carps form the backbone of inland aquaculture. Hatchery reared Mrigal (*Cirrhinus mrigala*) is often used to stock polyculture fish ponds throughout the country. The present study was designed to assess the genetic structure of Mrigal populations in hatcheries/farms and Rivers. Ten RAPD markers amplified 43 bands (mean, 6.14±0.50) to ascertain the genomic structure in hatchery reared and wild populations of major carp (*Cirrhinus mrigala*). Overall polymorphism varied with different levels from higher (69.77%) at all RAPD loci and with low among all populations (51.16%) of River Chenab. This was ascribed with the significant level of polymorphic information content. Mean values of genetic diversity ($h=0.2400\pm0.1524$) and Shannon's diversity index ($I=0.3815\pm0.2062$) for all populations of Mori fish were comparatively high varied from high (Manga Nursery Farm, 0.24±0.22) to low (Jaranwala Fish Farm, 0.16±0.15). DNA amplification showed different mean values of genetic diversity and genetic variance. Low level of genetic flow (mean, 2.8330±1.0731) among population showed the higher level of genetic variation among different populations of hatcheries/farms of *Cirrhinus mrigala*. On the basis of UPGMA of Nei, genetic distance ranged from 0.0009 to 0.0487 and dendrogram developed four main clusters, one comprising of C.F.S.H.Manawan and Fish Nursery Manga, second (River Ravi), third (Samundri, Jaranwala, Tandlianwala), and fourth with one group (River Chenab). This was resulted to low level of gene flow among populations for increase of genetic variation. Decreasing genetic diversity showed the high level of inbreeding coefficient within populations of hatcheries and farms that is ultimately affecting the growth rate of *Cirrhinus mrigala* in fish farms.

GENETIC VARIATION IN THE POPULATIONS OF *PRINIA INORNATA* USING DNA MARKERS

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Plain prinia (*Prinia inornata*) is an insectivorous a cosmopolitan species of birds found in Indus plains of Pakistan. It is a key species in agricultural ecosystem of the region. The present study was carried out to assess the genomic profile of *Prinia inornata* in southern Punjab (Bahawalpur and D. G. Khan districts) using RAPD DNA markers. A total of 102 (mean, 8.5±0.82) fragments/bands were amplified by applying 13 RAPD primers. Overall polymorphism (%) was

higher (75%) with different level in all RAPD loci and varied among all populations (low sites F; value 37.5%) to (high; site A & D; value 75%). Estimates of mean values of Shannon's diversity index ($I = 0.29 \pm 0.06$) and Nei's genetic diversity index ($h = 0.21 \pm 0.04$) for all populations were comparatively high and varied from high (site D, 0.4535 ± 0.2799) to low (site D, 0.3107 ± 0.1917). On the basis of UPGMA of Nei, the genetic distance ranged (0.0107 to 0.3984) and constructed dendrogram developed two main clusters, one comprising of four groups (Pop1 (A), Pop2 (B), Pop3 (C), Pop4 (D)) and second with two groups Pop5 (E) and Pop 6 (F). However, principle component analysis (PCA) endorsed genetic distances of UPGMA into six groups using three coordinates with variance of percentage i.e. 85.37, 95.05% and 3.00%. Overall high mean value of genetic variation ($Gst, 0.3238 \pm 0.0544$) were found among populations with decreasing of genetic flow in *prinia inornata* species. AMOVA analysis showed that the genetic variation within populations is higher 72% than among population i.e. 28%, which is the indicator of migration flow of bird populations between different sites in two areas. Furthermore the genetic differentiation was found significantly higher ($Fst, 0.312; p \leq 0.01$). This study of DNA markers can be used to find the effectiveness of conservation strategy to protect this species in the Pakistani range.

FREQUENCY OF CONSANGUINEOUS AND NON-CONSANGUINEOUS MARRIAGES IN DISTRICT SARGODHA, PUNJAB, PAKISTAN.

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Consanguineous marriages are observed among persons descending from the same common ancestor with close biological relations. Consanguineous unions are largely practiced and sometimes dominate in many populations of Pakistan. The present study was conducted to check consanguinity in district Sargodha of Punjab, Pakistan and inbreeding coefficient was calculated. The rate of consanguinity was calculated to be 56.72% and the inbreeding coefficient was 0.0348. The marriages between first cousins had the representation of 49.11% of all marriages and marriages up to distantly related are found to be 67.94% of all marriages. The hazardous health effects of consanguinity were also studied. When compared with other districts of Punjab regional heterogeneity was observed in the consanguinity pattern demanding further studies.

PREVALENCE OF ABO BLOOD GROUP IN DIABETIC NEPHROPATHY

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The present study was designed to study the relationship between blood groups and renal disease Diabetic Nephropathy. The study was done at Endocrine Unit and Diabetes Management Centre Services hospital, Lahore from January 2016-July 2016. For this purpose 540 samples were collected and divided into two groups, 203 control and 337 diabetic nephropathic subjects. Their blood groups were determined by a simple and widely used method of blood typing using Anti-A serum, Anti-B serum and Anti-D serum. Out of 337 diabetic nephropathic, 152 subjects were males

and 185 were females. The percentage of males and females in diabetic nephropathic group was 45.1% and 54.8% respectively. Smoking was prevalent in 22.2% of diabetic nephropathic subjects, 28.1% had family history of diabetic nephropathy and hypertension was present in 44.2% of them. The prevalence percentage of blood group A, B, AB and O in control group was 9.35%, 46.7%, 11.8% and 32.0% respectively and in diabetic nephropathic group 2.96% were in blood group A, 44.5% were in blood group B, 2.67% were in blood group AB and 49.8% were in blood group O. Blood group B was most common in general population and in diabetic nephropathic group blood group O was dominant. Blood group AB is the rarest group found in diabetic nephropathic subjects. In case of Rh factor, prevalence of Rh positive was found to be 92.6% in control group and in diabetic nephropathic group it was 94.9%. Rh negative prevalence in control group was 7.38% and in diabetic nephropathic group it was 5.04%.

GENETIC STUDY OF *SLC6A4* GENE IN CONVICTED OFFENDERS FROM PRISONS OF PUNJAB, PAKISTAN EXHIBITING ANTISOCIAL PERSONALITY DISORDER TRAITS

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Antisocial Personality Disorder (ASPD) is characterized by incapacity of an individual to adapt themselves to social norms. ASPD Patients typically have irritability problems and aggressive feelings toward other people. The serotonin transporter gene (*SLC6A4*) has been associated with regulation of serotonergic neurotransmission, mood and behavior traits. A 43bp deletion polymorphism in *SLC6A4* gene namely 5-HTTLPR polymorphism results in generation of a shorter allele S. This deletion of 43bp is associated with reduced transcriptional efficiency of the *SLC6A4* which consequently results in lower serotonin uptake activity leading to antisocial personality disorder. L allele (longer allele) has been associated with normal basal activity. Another SNP (Rs25531A>G) is exhibited at 1st base position of the deletion region so it is only present in L allele. This SNP (Rs25531A>G) has also been reported to be associated with decreased transcriptional efficiency of the *SLC6A4*. In current research, both the 5-HTTLPR polymorphism and Rs25531A>G polymorphism of *SLC6A4* gene were studied in criminal convicted for violent crimes as well as in control subjects to observe their potential association with Anti-social personality traits. S allele was found to have significantly higher frequency in convicted offenders as compared to the control subjects. Hence, it may be considered as a contributing factor in the development of the tendency to criminal's activity in our study population.

PREVALENCE AND SOCIO-DEMOGRAPHIC CORRELATES OF CONSANGUINITY IN DISTRICT MANSEHRA, PAKISTAN

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Consanguineous marriages, though practiced worldwide, are highly preferred in the Muslim countries and correlates with higher incidence of congenital genetic anomalies. This cross-sectional epidemiological study was carried out in district Mansehra of Khyber Pakhtunkhwa

province, Pakistan with the aim to elucidate the prevalence and socio-demographic correlates of consanguinity. Random data were collected on a designed questionnaire from a total of 1300 married female subjects through face to face interviews. Overall, 53.3% marriages in the district were found to be consanguineous with an inbreeding coefficient (F) of 0.028. First cousin unions were found to be most preferred type of unions (~70%). Tehsil-wise analysis of the data revealed highest consanguinity in tehsil Oghi (61.4%, $F = 0.034$) followed by tehsil Balakot (58.8%, $F = 0.031$) and tehsil Mansehra (48.2%, $F = 0.025$). Generally, prevalence of consanguineous marriages was found to vary significantly ($p < 0.05$) with respect to tehsils, urban/rural status, ethnicity, subject's spouse's occupation, number of persons per household, subject's literacy and parental consanguinity. Contrastingly, however, prevalence of consanguinity showed no significant difference ($P > 0.05$) when analyzed with respect to variables like subject's literacy level, number of rooms per household, house size and type, subject's age, marriages year and type, subject's language, origin, family type and occupation.

MOLECULAR CHARACTERATION OF *ZNT* REGULON FOR MULTI-METAL RESISTANCE IN *KLEBSIELLA PNEUMONIAE*

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Metals at high concentration are toxic to microorganisms. A chromosomally encoded *znt* operon plays an important role in multi-metal resistance in some members of Enterobacteriaceae. However, its role has not yet been explored in *K. pneumoniae*. This operon comprises two putative genes *i.e.* *zntR* and *zntA*. The minimum inhibitory concentration (MIC) of lead, zinc, mercury and cadmium was proved to be in the order of $Pb > Zn > Cd > Hg$ against *K. pneumoniae*. The metal uptake and storage ability of this strain was assessed through atomic absorption spectroscopy. *zntR* gene was amplified and cloned in pTZ57R. ZntR protein was expressed in pET expression system and purified through Fast Protein Liquid Chromatography (FPLC). Determination of role of these genetic elements for metal resistance in *K. pneumoniae* and exploration of resistance mechanism involved at molecular level may open new horizons of knowledge that would be helpful in devising bioremediation and biomining strategies in future.

CASE REPORT: ESOPHAGEAL DIVERTICULUM IN A CAT

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A domestic female cat of persian breed having 10-year age was presented with the complain of improper regurgitation, panting after eating, very sudden weight loss, increased pulse and respiration rate at pet centre Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan. Prior to 08 months of onset of these clinical findings, the cat had received nutritional supplementation through nasogastric tubes and gastrostomy. With the help of clinical findings, endoscopy and contrast esophagram, it was diagnosed as esophageal diverticulum and tube feeding

complication. Treatment with the parental fluid therapy, metaclopramide, cisapride and ranitidine supplemented with a soft diet containing low fat contents has shown major improvement in the clinical picture within 02 weeks. Recovery status was confirmed by follow up with owner of cat.

3. HUMAN AND ANIMAL DISEASES

INCIDENCE OF MACULAR CORNEAL DYSTROPHY IN PUNJAB POPULATION

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Macular corneal dystrophy is an autosomal recessive hereditary disease in which slowly progressive punctate opacities in the cornea result in bilateral loss of vision, eventually necessitating corneal transplantation. A retrospective, analytical study was performed between October 2015 to June 2016 on Macular corneal dystrophy patients visiting LRBT, General Hospital, Al-Ehsan welfare Hospital, Mughal eye and Mayo Hospital during their ophthalmic consultation. The aim of this research was to determine prevalence of Macular corneal dystrophy in the families of Pakistan. The techniques used for the diagnosis of MCD were visual Acuity test by Snellen chart, phoropter, slit lamp biomicroscopy, topography, keratometer and pachymetry. In this study, 50 patients of MCD were identified among which 40 were males and 10 were females, including 9 cases with family history. Main complaint was drop in visual acuity and loss of vision in all patients (100%). 87% cases have high vision loss and 13% patients have moderate vision loss. MCD were bilateral in all cases (100%). Cases of MCD were observed in all age groups but 40% cases was found in age 21-30 years. Corneal thickness was decreased in 73.8% cases due to accumulation in corneal stroma. Careful clinical evaluation, early diagnosis, genotyping, genetic counselling and proper treatment (corneal grafting) are necessary for the restoration of optimal vision and should be done to avoid the severe outcomes caused by MCD.

PREVALENCE OF ANEMIA IN SCHOOL AGE CHILDREN (SAC) OF DISTRICT SWABI, KPK, PAKISTAN Mushtaq

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Although swabi has proficient rapid economic growth over the decades, significant health and nutrition problem remains unfortunately because little work has been done to track basic diseases such as anemia; exact prevalence of these health problems is often unknown. The goal of this research is to assess the prevalence of anemia in swabi. A total of 400 children of school age participated in this study. District swabi was divided into four tehsils topi, swabi, razzar and lahor and 100 samples of blood of both genders were collected from each subject to measure hemoglobin level. From tehsil Swabi 60 boys and 40 girls, tehsil Topi 70 boys and 30 girls, Razar 50 boys and 50 girls and Lahor 80 boys and 20 girls, were examined in the current study. Children included in the study sample were divided into two groups, anemic and non-anemic. In tehsil Swabi 42% of school going children were anemic. The prevalence of anemia in school going children of tehsil Topi was 48%, in tehsil Razar the count of anemic children was 63% and 58% children found anemic in tehsil Lahor. The prevalence was high in tehsil Razar, Lahor, Topi and Swabi respectively. The total anemic children were 52.0% and non-anemic were 48.0%. The mean Hb level of anemic children was 10.9 gm/dL.

DENGUE FEVER VIRUS: ASSOCIATED FACTORS AND TREATMENT THROUGH RECOMBINANT DNA TECHNOLOGY

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Dengue is a wide-spread mosquito-borne viral disease concern with over two-fifths of the world's population at risk of infection. Clinical presentation may vary from undifferentiated fever, classic dengue fever, dengue hemorrhagic fever to dengue shock syndrome. The risk of severe disease is much higher in sequential rather than primary dengue infection. Usually clinical suspicion for dengue fever is sufficient for supportive treatment. Complete blood picture may show high hematocrit, leucopenia and thrombocytopenia. Complete blood count is the most common diagnostic tool to study the dengue patients in which there is a fall decrease in the platelets and total leukocyte count. Although intensive research is being carried out to investigate the underlying mechanisms causing severe dengue but a fundamental knowledge gap still remains to be filled regarding host cell tropism, crucial host immune response mechanisms, and viral markers for virulence. Despite the fact that search for a suitable dengue vaccine has been ongoing for the last sixty years, yet any effective vaccine or treatment remains elusive. A vaccine must be protective for all four serotypes of dengue and should be cost-effective too. Many approaches have been employed to develop candidate vaccines till date. These include live attenuated tetravalent vaccines, chimeric tetravalent vaccines based on attenuated dengue virus or Yellow Fever 17D, and recombinant DNA vaccines which target flavivirus and non-flavivirus vectors, some of which are at the stage of clinical testing. This review outlines the progress that is being made in the development of a vaccine, and the challenges involved in dengue vaccine development. The current stages of proposed vaccine candidate development will also be presented.

SEROPREVALENCE OF TOXOPLASMOSIS IN FEMALE POPULATION OF LAHORE, PAKISTANASMA ABDUL LATIF, RAFIA TABASSUM, SABIHA FAZAL,
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Toxoplasmosis is a wide spread zoonotic infection of warm blooded animals including humans all over the world. This infection is caused by a Protozoan parasite *Toxoplasma gondii*. The parasite completes its life cycle in both humans and cats. It causes severe congenital abnormalities such as hydrocephalus and mental retardation in infants. Most *T. gondii* infections among humans occur by eating raw or undercooked meat containing *T. gondii* tissue cysts, by ingesting oocysts from soil, or by acquiring congenital infection through the placenta of mother. Keeping in view the importance of this parasite, the present study was designed to study the seroprevalence of toxoplasmosis female population of Lahore, Pakistan. Fresh blood of females were collected and serum was centrifuged and analyzed to estimate the seroprevalence of toxoplasmosis by using ELISA technique. All the information was collected with the help of questionnaire and analyzed to find out the risk factors. Overall prevalence in female population in Lahore was found 27%. Among pregnant and non pregnant females prevalence rate was 31% and 24% respectively. It was observed that prevalence of *T. gondii* increases with increasing age.

Women who experienced abortion had high prevalence rate (66.6%) as compared to normal pregnant females (25.6%). Prevalence rate was higher in those females who are housewives (33.3%) as compared to workers (25%). because of direct contact with vegetables during food preparation and had contact with cats or any pet animal.

COMPARATIVE STUDY OF RELEVANT CIRCULATORY PROTEINS BETWEEN NON INSULIN DEPENDENT DIABETES MELLITUS (NIDDM) AND NORMAL FEMALE LOCAL POPULATION

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An experiment was carried out between three age groups (1:30-39, 2:40-49, 3:50-59 years) to compare blood protein fractions of NIDDM and normal female local population. Blood samples were collected containing anticoagulant (EDTA) and enzyme inhibitor (NaF) to inhibit glucose utilization by blood cells. Extracted plasma processed for glucose estimation and the same blood samples were placed for serum separation. Biochemical analysis revealed the high glycemic level and lower total proteins and serum albumin in diabetic subjects with advancing age. Serum globulins and non gamma globulins reported lower and gamma globulins slightly higher in diabetic group as the fractions increased significantly with respect to age. Free amino acid content evidently lower in controlled population comparing diabetic category. The described outcomes revealed that local female population exhibit non significant variations between NIDDM and normal categories with reference to the protein contents.

ROLE OF IRISIN IN THE DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME: A COHORT STUDY AMONG FERTILE AND INFERTILE PCOS WOMEN

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Irisin is a novel, myokine involved in modulation of energy expenditure by mediating its beneficial effects on exercise by transforming white adipose tissue to brown adipose tissue. Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by ovulatory dysfunction, hyperandrogenism and polycystic ovaries that leads to insulin resistance and obesity. The present study was design to investigate the diagnostic role of irisin in healthy women and women having primary and secondary infertility with PCOS, correlated with anthropometric, metabolic and reproductive hormonal parameters. Eighty five female subjects (21-42 years) including healthy women (n=15), primary infertility with PCOS (n=42) and secondary infertility with PCOS (n=28) were enrolled in this study. Blood samples were obtained and analyzed. Plasma irisin concentration was determined by Enzyme Immunoassay Assay (EIA). A significant decrease ($p < 0.05$) in irisin concentration was observed in women with primary infertility with PCOS while non significant decrease in secondary infertile with PCOS women as compare to healthy women. Similarly, significant increase ($p < 0.05$) in body mass index, LH, T, prolactin and glucose concentrations, while non significant increase in TSH and T₃ was noticed in PCOS when compared

with healthy subjects. Additionally, a significant decrease ($p < 0.05$) in FSH and T_4 was observed in case of PCOS. Therefore it is concluded that reduced level of circulating irisin might be used as predictor of PCOS as well as stronger predictor of glucose tolerance and hyperandrogenism in females, so irisin can be used as a biomarker for diagnosis of PCOS thus facilitating early detection and treatment to reduce infertility.

CHARACTERIZATION OF HEPATITIS B VIRUS GENOTYPES IN CHRONIC HBV PATIENTS OF DISTRICT BANNU KHYBER PAKHTUNKHWA, PAKISTAN

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Hepatitis B Virus infection is a global health problem with its continuously increasing burden on the developing countries like Pakistan. Pakistan falls in the highly endemic region for HBV. The present study was conducted with the main aim to characterize various HBV genotypes in chronic HBV patients of district Bannu. A total of 208 blood samples were collected from HBV suspected individuals of either sex, with mean age 31.42 ± 16.95 years, ranged from 1-70 years. All the subjects were screened with Immunochromatographic test (ICT), for HBsAg. The positive patients for HBsAg were processed for the detection of HBV DNA by nested PCR and HBV DNA positive patients were subjected for HBV genotyping by genotype specific PCR. Complete Blood Count (CBC) and Liver function tests (LFTs) were performed for every individual and were compared with particular HBV genotype. Data obtained was analyzed with statistical software SPSS. A total of 208 blood samples were collected from the HBV suspected individuals among which 100 patients including 77 males and 23 females were found HBsAg positive and were processed for the study with age 1-70 years, including all the areas of Bannu Division. The remaining 108 patients (72 male and 36 female) were remain negative for HBsAg and were excluded from the study. These samples were collected from the Hepatitis Diagnostic Laboratory, District Head Quarter Hospital Bannu, where the HBsAg suspected patients were referred to by the hospital physicians. Of the total HBsAg positive analyzed patients, 83 were confirmed for HBV DNA, including 66% (66/100) males and 17% (17/100) females, while 17 patients including 11% (11/100) males and 6% (6/100) females were found negative for HBV DNA by PCR (Table 4.6). Among the total 83 HBV DNA positive samples, 80 (96.38%) were classified for genotypes by showing genotype specific bands for genotype A, D and mixed genotype A+D. These include 65 (81.25%) males and 15 (18.75%) females. The predominant genotype in this area in HBsAg positive patients was genotype D 40% (40/100), followed by genotype A 30% (30/100), and the mixed genotype (A+D) were found in 10% (10/100) individuals while 3% (3/100) samples, including 1 male and 2 females were remain untypable. No other HBV genotype was found in this area of Bannu Division. Percentage of neutrophils, lymphocytes, eosinophils and monocytes were found to be normal in all the subjects. Similarly, hemoglobin level was found to be normal in all the subjects. Risk factors for HBV were sharing personal items, shaving in barbers shops, blood transfusion, dental risk, general surgery, history of injection, sexual contact with HBV positive partner and skin tattooing. It is concluded that genotype D and A are circulating in the study area with the most prevalent genotype D. Comprehensive studies regarding HBV genotyping and proper treatment and vaccination should be initiated to stop the fatal disease.

THE GENETIC AND PUBLIC HEALTH ANALYSIS OF EYE MALFORMATIONS IN SUKKUR REGION OF SINDH-PROVINCE, PAKISTAN

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Heredity material in the form of genes transmitted from parents to their offspring. Most of the genetic diseases are transmitted due to the lack of knowledge and socio-economic culture. Prevalence of consanguineous marriages is the main cause of the transmission of mutant genes generation after generation. Eye disorders are the congenital disorders which became a main public health problem of the world. Malformation of eye means "an abnormal formation of eye". People of Sindh practice consanguineous marriages from years and years due to which, their progeny shows a lot of genetic disorders generation after generation. The study on the public health and genetic analysis of eye malformations was done in Sukkur region where the previously done work was not found in literature in which the affected individuals of the eye malformations were seen with public health perspectives by using different methods as; Survey using KAP and cross sectional studies, Pedigree records, and the diagnosis and Hospitalization from concerned medical consultants. After non-random sampling, the cross sectional survey of the 132 different villages of the Sukkur region was done using KAP method in which 20 families with genetic eye malformation were searched. 14 out of 20 families were selected for the study. The public health disorders seen are such as Achondroplasia, Muscular dystrophies; Neurological disorders, Schizophrenia and Reproductive disorders etc were seen in the families. Each pedigree of 14 families shows the generations with multiple public health diseases including the congenital eye malformation. Diagnosis of 38 patients of 14 families was done in LRBT Gambat where 09 patients were hospitalized for surgery of congenital cataract while as 03 Children were recommended to LRBT Karachi for surgery due to the lack of presence of EUA in LRBT Gambat. Most of the people of all villages were not aware of the mode of diseases transmission. Hence, knowledge about genetics and public health awareness was also provided to them. The next steps in the study are the blood collection of the affected individuals of genetic eye malformation for DNA extraction to process the molecular techniques for finding out the different mutant genes in the DNA.

ANALYSIS OF THYROID DISORDER (HYPOTHYROIDISM) IN BOTH GENDER FROM DISTRICT JAMSHORO SINDH

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Thyroid disorder is related to hypothyroidism, When thyroid gland released too little thyroid hormone, which cause swelling in neck, chest pain ,hypertension ,weight gain ,depression etc. During the study period 2016, we assessment with the help of laboratory investigation include whole thyroid profile TSH, T3 and T4.Serum Aliquots technique was used . That the 204 patients were suffering from hypothyroidism. Out of 204 patients 48 were male and 156 were female patients .We found hypothyroidism is more common in female than in male in district Jamshoro .

EXPRESSION OF GSST1 IN TYPE 2 DIABETIC RETINOPATHIC SUBJECTS

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Diabetes is one of the multifactorial disorders with genetics and environmental factors playing important role in its cause. Type 2 Diabetic Mellitus (T2DM) is the most common metabolic and multifactorial disease in which both genetic and environmental factors are involved. In diabetes, the defects in cellular metabolism results in increasing free radicals. These radicals react with other vital cellular molecules which are responsible in diabetes side effects and known as Diabetic Retinopathy (DR). Human glutathione S-transferases (GST) are a family of enzymes that catalysis conjugation of electrophilic substances with glutathione. The aim of present study was to investigate the expression of glutathione S-transferases class Theta 1 (GSTT1) in type 2 diabetic retinopathy subjects. The subjects were sampled from Fatima Memorial Hospital and Sheikh Zayed hospital of Lahore. The studied subjects were divided into three groups Diabetic retinopathy (n=25), diabetic (n=25) and healthy control subjects (n=25). Subjects with kidney infection, cardiac failure, tumors, cancer, kidney dialysis, Inflammatory, infectious and autoimmune diseases were not included in this study. The patients were identified by ophthalmologist. A self-designed questionnaire was filled by each participant to collect the data regarding age, socio-economic status, exercise and disease history. Results were analyzed statistically by SPSS. Diabetic Retinopathy (DR) was more prevalent in females. RNA was isolated from studied subjects. After quantification of RNA by Nano drop, first strand cDNA was prepared by using Thermo kit. The gel electrophoresis was performed to detect the confirmation of cDNA. The expression analysis was performed by Real Time PCR to investigate the expression of GSTT1 in T2DR patients. Highly significant differences (P<0.05) were observed in GSST1 expression in diabetic retinopathy patients as compared to diabetic and control groups. Hence, it is concluded that the proportion of the expression of GSTT1 was significantly greater in diabetic subjects when compared to controls which are responsible for increasing the risk of diabetic retinopathy.

INCIDENCE TRENDS OF TUBERCULOSIS: AN INTEGRATED STUDY OF UNDERPRIVILEGED CHILDREN IN AZAD JAMMU AND KASHMIR, PAKISTAN

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Tuberculosis is a disease affecting the respiratory tract of its victims and is found mostly in low socio economic segment of society. The current research was done to evaluate the trends in

tuberculosis infection among the underprivileged children (orphans) in Azad Jammu and Kashmir (AJK), Pakistan. Study included 1708 children, which were analyzed during four years. First round of screening was carried out in 2011 involving 733 children from seven districts of AJK and three years later, 975 unrelated children were investigated. The antibody positive subjects were subjected to sputum smear and PCR test to confirm the active tuberculosis. Major risk factors and values of plasma markers in diseased participants were also assessed. In 2011, an overall 5.18% seroprevalence and 3.41% active tuberculosis was found. Females with 3.13% seroprevalence and 2.05% active infection indicated higher infection rate as compared to the male children by 2.05% and 1.36% respectively. In 2014, we found 2.77% and 1.95% antibody positive and active tuberculosis cases respectively. The infected females included 1.64% and 1.12% and males included 1.13% and 0.83% positive for antibody and active TB. The infected children had an overall elevated plasma values for lymphocytes, granulocytes, relative volume of platelet PCT%, mean corpuscular hemoglobin concentration MCHC and red cell distribution width RDW. However, a decrease was observed for mean corpuscular volume MCV, haematocrit HCT, blood calcium and body mass index of infected subjects. A general decline in tuberculosis found during four years can be associated with the improvement in the overall living and medical facilities in the recent years. An overall replacement of serological antibody based diagnosis is recommended in Pakistan.

THERAPEUTIC EFFECT OF QUERCETIN IN LETROZOLE INDUCED POLYCYSTIC OVARY USING RAT MODEL: A HISTOLOGICAL AND A BIOCHEMICAL STUDY.

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In the present study, therapeutic effects of quercetin were investigated in a rat model of polycystic ovarian syndrome. Twenty four female Sprague Dawley (SD) rats were divided into four groups (n=6); control group received aqueous solution of carboxymethyl (CMC 0.5%); PCOS group administered with letrozole (1mg/kg) dissolved in solution (CMC 0.5%); Metformin group given metformin (20mg/kg) along with letrozole (1mg/kg); and Quercetin group provided with quercetin (30 mg/kg) along with letrozole (1mg/kg). All doses were given orally via gavage for 21 consecutive days. The PCOS group exhibited prolonged diestrous stages, increased levels of testosterone and decreased levels of estradiol and progesterone. The body weight, mean ovarian weight and ovarian diameter were reduced ($P < 0.001$) in metformin and quercetin treated groups as compare to PCOS group. Letrozole induced PCOS rats showed adverse ovarian histopathology including presence of cysts, reduced granulosa and theca layer, decreased number of healthy follicles and increased number of corpus luteum and atretic follicles. In contrast, these adverse effects were normalized by co-administration of quercetin metformin. Moreover, the hormonal concentrations of estradiol and testosterone were restored to the optimal levels. In conclusion, quercetin significantly diminished the harmful clinical and biochemical fluctuations in letrozole induced PCOS rat model and therapeutic effects of quercetin were comparable to metformin.

ACHONDROPLASIA, A HIDDEN GENETIC DISORDER EXPLORED IN SUKKUR REGION SINDH - A PIONEER CROSS SECTIONAL STUDY IN PAKISTAN

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Achondroplasia is the commonest form of skeletal dysplasia leading to dwarfism with associated Oral health and general health issues, a type of skeletal dysplasia occurring in approximately 1 in 25,000 live births. It is an autosomal dominant condition, caused by a mutation in fibroblast growth factor receptor 3 (FGFR3) on human chromosome 4. Its a common observation for this genetic disorder to be commonly found In Sukkur region in the local communities. The local literature was reviewed thoroughly but, the researcher could not find any published work on this disorder in Sindh Province, esp. in Sukkur region so this topic was selected by the corresponding author for the study. The survey was done using KAP questionnaire method followed by the pedigree charts making. Finally the traced individuals of Achondroplasia were diagnosed on the basis of its characteristic clinical and radiographic findings. The results show that among the total number of 25 families, 06 were found affected with average 3-7 patients per family. The disease transmission test was also done statistically. The study patients blood was collected for further genomic analysis using PCR and other molecular laboratory techniques, after informed consent. The abstract of this pioneer local study show that, more studies are needed to further explore its associated oral and general health and their social issues in Pakistan.

PREVALENCE OF β - THALASSEMIA IN CHILDREN OF MUZAFFARABAD DISTRICT OF AZAD JAMMU AND KASHMIR.

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Thalassemia major is one of the most common genetic disorders in Pakistan and over five thousand new patients are added in the pool annually. This familial disease has both medical and social implications, and therefore there is a need to assess the magnitude of Thalassemia trait. This cross-sectional descriptive study enrolled 252 subjects from Thalassemia Center CMH Muzaffarabad, which had thalassemia like symptoms. 71(28.2%) subjects were found thalassemic after diagnostic analyses of thalassemia (including 35(49.3%) male and 36(50.7%) female). Beta-thalassemia was predominant as compared to Alpha-thalassemia because all patients were β - thalassemic, no Alpha-thalassemic patient was found. Out of 71 patients, 32 patients belonged to age group 1-5 years, including 16 males and 16 females. 22 patients belonged to age group 6-10 years, which was consist of 11 males and 11 females. Similarly the third age group was 11-15 years

having 16 patients, out of which 07 were males and 09 were females. The last age group was ranging from 16-20 years. This includes only 01 individual who was male, no female was found thalassemic in this group. In Muzaffarabad city out of 71 total thalassemic individuals, 28(39.4%) thalassemic individuals were found that is the highest prevalence from all other regions. The lowest percentage of thalassemic prevalence was found in Patika in which 10(14.1%) individuals were found thalassemic. In the present study out of 71 total thalassemic individuals, 23.9% patients belonged to Rajpoot tribe which is highest prevalence among all other casts. The lowest percent prevalence was found in Sheikh Tribe which was 2.8%. 84.5% affected individuals belonged to cousin married parents while 7% were belonging to that parents which were relatives but not cousins. Prevalence of thalassemia was also found in individuals whose parents was non relative. The percentage of this type of affected individuals was 8.5%. Our findings strongly suggest screening and genetic counselling for β -Thalassemia trait in families of Thalassemia major patients of Azad Kashmir.

**INCIDENCE OF HEPATITIS B IN THE STUDENTS AND EMPLOYEES OF
UNIVERSITY OF AJ&K MUZAFFARABD.**

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Hepatitis B have become very lethal and rapidly increasing infectious diseases of Pakistan. It is affecting millions in the country and in order to devise a strategy to halt its ever increasing disease, burden the current study was designed to know the exact status in the population of AJ&K University. Recent study was designed for screening, awareness and treatment of hepatitis B of students and employees of University of Azad Jammu and Kashmir Muzaffarabad. Methodology was based upon department-wise awareness seminars, basic information collecting proforma's, knowledge assessment questionnaires, blood sampling and serum separation, screening for HBsAg through ICT Kit method, ELISA based confirmation of ICT positive samples and RISK factor assessment through questionnaire. A total population of 7015 was screened for HBsAg by using ICT Kit method. 150(2.13%) individuals were found positive for HBsAg after initial screening through ICT Kits. 117(1.7%) individuals were confirmed through ELISA. The age group 21-26 is found more affected among all age groups which includes 78(66.7%) of 117 ELISA confirmed positive HBsAg individual. District Muzaffarabad is more prevalent among all districts, it resides 60(51.3%) HBsAg ELISA positive individuals. Dental Surgery and Mourning Blades were the most pre-dominant risk factors among all the HBsAg positive individual. Awareness of more than 300,000 people belonged to different communities was carried out through conduction of awareness sessions. 1.7% prevalence of hepatitis B among the highly educated community is an alarming health concern regarding the ever increasing spread of deadly hepatitis B in Pakistan.

**INCIDENCE OF HEPATITIS C IN STUDENTS AND EMPLOYEES OF
UNIVERSITY OF AJ&K MUZAFFARABAD**

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Hepatitis C remains and uncontrolled health problem of the recent era. The present study was aimed at finding the prevalence of hepatitis C in the total population (students and employees) of University of Azad Jammu & Kashmir Muzaffarabad to save their lives from the silent killer. The studied population was apparently healthy and majority belonged to active and energetic age group. Sampling campaign continued for three months in university. All departments of university were bounded for screening of their individuals by executive orders of higher authorities of university. Conduction of seminars about the awareness of disease and its prevention was done in all departments. Blood samples were taken by venous puncture and serum was used for detection of anti-HCV antibodies by Enzyme Linked Immuno Sorbant Assay (ELISA). Out of 7015 individuals (45.77% males and 54.22% females) Hepatitis C virus antibodies were found in 65 individuals showing 0.92 % prevalence. Males showed 1.34% and females showed 0.58% prevalence. On the basis of age groups, 82.22% population was from 16-25 year old age group, 10.47 % were from 26-35 group, 3.9% from 36-45 group, 2.75% were from 46-55 group, 0.65% were from 56-65 group showing 0.67%, 1.77%, 0.72%, 5.69% and 2.17% prevalence respectively. On the basis of geographical distribution, 55.82% population was from district Muzaffarabad, 8.11% from Bagh, 6.54% from Hattian, 5.4% from Neelum, 4.73% from Poonch, 2.05% from Haveli, 2.02% from Sudhnoti, 1.71% from Punjab and 0.099 % from Federal Area showing 0.69%, 1.58 %, 1.3%, 1.31, 3.01%, 0.69%, 1.4% and 14.28% prevalence respectively. The presence of disease in the highly educated and well off segment of society indicates a manifold vulnerability of the risk groups of catchment populations of the State of AJ&K.

**EPIDEMIOLOGICAL ESTIMATES OF TYPE 2 DIABETES MELLITUS REPORTED IN
A HOSPITAL POPULATION OF FAISALABAD**

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Epidemiological study was performed to estimate the incidence, prevalence and risk factors responsible for T2DM and using a standardized questionnaire for hospital patients at Allied and District Headquarter Hospitals, Faisalabad. Data was statistically analyzed for percentage (%) distribution, average, chi-square test (χ^2 test) and coefficient of inbreeding (F). It was found that type 2 diabetes mellitus was the most common type (74.58%) followed by type 1 diabetes mellitus (25.42%). Out of 1015 subjects of diabetes 589 (58.02 %) were male and 426 (41.87%) females. Average age of diagnosis was 44.67±0.44 years and average age at present 54.38± 0.49 years. Age group 41-50 years was more prone to this disease (33.99%). Most of the patients were married (98.33%). There were 54.67% urban and 44.73% rural diabetic patients. Diabetes found to be more prevalent in the retired / jobless patients (40.59%) with school level education (59.9%) with middle socioeconomic status (92.31%). The information regarding smoking status revealed that there were

smokers (26.99%), non-smoker (71.43%), ex-smokers (1.58%) and passive smokers (31.92%). Effect of lifestyle, dietary habits, choices and preferences in diabetic patients showed that 98.7% had mixed type of diet while majority of the patients belonged to fresh and spicy food category (98.32%). Majority patients were not used to have exercise (72.8%) and walk (50.54%) and 43.25% were obese subjects and 31.43% were with 41-45 inches waist measurements. Associated complications were observed including heart diseases (13%), hypertension (11.72%) and liver problems (6.90%). Majority of subjects (51.43 %) were facing stressed conditions, 44.04% high blood pressure and 4.14% low blood pressure. Weak eye sight was a common problem in diabetic patients (61.18%). This study would be helpful in suggesting certain strategies to reduce its prevalence rate in Pakistan.

**UNDERPRIVILEGED YOUNG POPULATION OF AZAD JAMMU AND KASHMIR,
PAKISTAN UNDER THE THREAT OF VIRAL INFECTION HEPATITIS B AND C:
AN ALARMING HEALTH CONCERN**

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Hepatitis B and C pose a major threat to the health of low socio economic people of the society. The aim of this study was to record the prevalence of viral hepatitis B and C in the under privileged population of AJ&K. Under privileged orphans of age 5-17 years in three districts Hattian Bala, Kotli and Poonch of Azad Kashmir, Pakistan were included in this study. The selected population was appeared to be underprivileged orphans in terms of low socioeconomic status, low education of guardians, poor housing facility, etc. For primary screening of anti-HCV and anti-HBsAg, a total of 573 individuals were selected and screening was performed using commercial kits. The positive cases were confirmed by qualitative nested PCR of Hepatitis B Virus (HBV) DNA and Hepatitis C Virus (HCV) RNA. Moreover, viral load of HCV RNA was estimated by Real-Time Quantitative PCR. The study was further elaborated by assessing the risk of infectious diseases. During surface antigen screening, only one individual (0.17%) was found positive for hepatitis B surface antigen while a total of 68 (11.86%) individuals were found positive for anti-HCV antibodies. Furthermore, 15 individuals were found positive for hepatitis C RNA after nested PCR. In genotype distribution analysis, the most frequent genotypes were 2a and 3a among this population. Moreover, different risk factors i.e., blood transfusion, surgery, dental treatment, injection, etc. were evaluated and this study concluded that the most of the people visited the clinics of unqualified medical practitioner. Our study could help to undertake the systematic surveys at a larger scale and to devise ways and means to eradicate the deadly infectious diseases from the society of Pakistan.

STUDY OF THE EFFICACY OF CADMIUM- ROS-MEDIATED PHOTODYNAMIC THERAPY ON HUMAN RHABDOMYOSARCOMA (RD) CELL LINEABDUL WAHEED¹, BUSHRA MIRZA¹ AND MUHAMMAD RAFI²¹*Department of Biological Sciences, Quaid - I - Azam University, Islamabad*²*Pakistan Institute of Engineering and Applied Sciences P.O. Nilore, Islamabad*

Photodynamic therapy (PDT), a newly emerging technology for cancer treatment is extensively studied. We designed a set of systematic experiments to study the efficacy of PDT in vitro. A cell line and photosensitizer (PS) were used during this study to evaluate untargeted PDT outcome. Initially photosensitizer based PDT efficacy was investigated on human skeletal muscle cancer rhabdomyosarcoma (RD) cells in vitro. The toxicity of laser light itself and the PS alone to these cells were found to be negligible. The incubation time, PS concentration and light doses were optimized for RD cell line. The cell viability of RD was reduced to 30-40 % when exposed to optimum parameters of PS and light doses. Cadmium(Cd), a heavy metal, which has a potent harmful effects, is a highly stress-inducible material that is robustly expressed following disruption of homeostasis in the endoplasmic reticulum (ER) (so-called ER stress). The mechanism Cd induced cell death of RD cells complex, involving cellular signaling pathways as yet incompletely defined but, in part, involving the generation of reactive oxygen species (ROS). Several studies have correlated GADD153 expression with cell death. RD cells treated with cadmium chloride led to increase in intracellular ROS levels in a doses and time dependent manner. The cell viability of RD was reduced to 35% when exposed to optimum parameters of cadmium chloride.

PREVALENCE OF MALARIAL DISEASE IN LOCAL POPULATION OF TEHSIL BARIKOT, SWATMAZHAR ALI¹, MUZAFAR SHAH², SARDAR AZHAR MEHMOOD¹, MIAN SAYED KHAN³,
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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.

PREVALENCE AND EPIDEMIOLOGY OF *ACNE VULGARIS* IN FAISALABAD, PAKISTAN

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The present research work was conducted to study the prevalence and epidemiology of *Acne vulgaris* in humans in Faisalabad through a standardized questionnaire, which was prepared under the supervision of certified skin specialist. One hundred subjects were examined and information recorded in the questionnaire. The data was analyzed by applying statistical analysis (Minitab, Crosstab, Pearson Chi Square test and Analysis of Variance). The results of present study showed that the teenagers (16-20 year) were mostly affected (59%). Females were affected (59%) which was more than males (41%). 24% spicy food is used by people but 72% people had no aggravating effect while 28% people had this. 58% people had no gastric problem but they had acne vulgaris. 57% females in which 25% involved in premenstrual flare and 14% in postmenstrual flare played a significant role in increase of acne vulgaris. 82% people had oily skin. 40% Dust, 7% smoke particles and 7% sunlight caused the acne vulgaris. 16% got this disorder from parents but 82% no this disorder. 31% people had previous disease (leukaria, headache, pile) and 69% had no previous disease. 87% people used allopathic and 13% Homeopathic. 56% Acne vulgaris appeared on face. The oily skin type was found more vulnerable to this disorder. Acne vulgaris is caused by these two bacteria mostly *Staphylococcus epidermidis* and *Propionibacterium acnes*. Females with pre-menstrual and post-menstrual flare played a significant ($P < 0.01$) role in increase of acne vulgaris.

CLINICAL CHARACTERISTICS OF PATIENTS WITH HEPATOCELLULAR CARCINOMA IN SUBURBAN RAWALPINDI, PAKISTAN

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Hepatocellular carcinoma (HCC) is the most common primary liver tumor and represents the third-leading cause of cancer-related death in the world. The incidence of HCC continues to increase worldwide, with a unique geographic, age, and sex distribution. The present study is aimed to determine prevalence of hepatocellular carcinoma (HCC) in sub-urban Rawalpindi Pakistan and

to evaluate the role of its associated risk factors. The study was conducted from August 2015 to July 2016. Blood samples (2-3 ml) were collected from diagnosed patients of HCC along with the negative control that include healthy subjects. The aspirated blood was shifted to silica gel tube for biochemical analysis. Blood was centrifuged for the separation of the sera. The serum was stored at -80°C for further analysis. Biochemical profiling was performed. Blood was continued for the liver capacity test, lipid profile and hematological files. All tests were performed utilizing industrially accessible reagent units Current result showed that those people with the age between 30-60 years were potentially at higher risk of getting HCC. Females are at higher risk of getting HCC than the males (P<0). Clinico-pathological results showed that including higher value in HCC patients than the normal NON-HCC people.

CHARACTERISTICS AND RISK FACTORS OF HIRSUTISM AMONG FEMALES OF RAWALPIDI-ISLAMABAD

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Hirsutism is a condition of unwanted, excessive growth of stiff male pattern hair among women that may arise due to excess of androgens, primarily testosterone. Unfortunately, it has never been taken as disease and no serious efforts are underway to estimate the gravity of risks and its threat to female health. Therefore, the present study was conducted to evaluate the characteristics and risk factors of hirsutism among the females of Rawalpindi and Islamabad. A survey was conducted to collect data from general female population (N=6500) of Rawalpindi and Islamabad. Prevalence of hirsutism among females of Rawalpindi and Islamabad was 5.6%. Information relevant to characteristics and risk factors was collected from clinically diagnosed 117 hirsute females visiting Gynecology Department of Benazir Bhutto Hospital Rawalpindi, and Gynecology and Dermatology Department of Pakistan Institute of Medical Sciences (PIMS) Islamabad. The data were analyzed using Chi Square (χ^2) and simple and multiple logistic regression using SPSS software. The most common symptom was thinning of scalp hair that was significantly (P<0.05) high among patients (86%) compared to normal females (9%). The signs of virilization such as voice deepening (14.5%) and clitoris enlargement (12.8%) were observed in patients while these characteristics were not observed in normal females (P<0.05). The appearance of terminal hairs was highest on chin (94%) followed by upper lip and lower abdomen (73%), near nipples (49.57%), chest (36.75%), upper abdomen (17.09%), lower back (15.38%), and upper back (12.8%). The major risk factors of hirsutism include plucking (AOR 3791.885 times), consumption of broiler chicken (AOR 2513.933 times), oral hormonal medication (AOR 184.485 times), acne (AOR 112.681 times), being married (AOR 70.493 times), being overweight/obese (AOR 26.458 times) and sub fertility (AOR 12.000 times) with 95% confidence interval (P<0.05). This study reports that chins (94%) is most sensitive to terminal hairs among all selected body parts. Hair plucking, consumption of broiler chicken, oral hormonal medication, being overweight/obese are major avoidable risk factors of hirsutism among the females of Rawalpindi-Islamabad.

**RISK FACTORS AND COMPLICATIONS OF MENORRHAGIA AMONG
FEMALES OF RAWALPIDI-ISLAMABAD**

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Menstrual cycle is the reproductive cycle in human females with entire blood loss between 25 and 80 ml lasting an average of 5 days. Menstrual flow lasting longer than 7 days with a blood loss of greater than 80ml constitutes menorrhagia. Present study was conducted to check the epidemiology of menorrhagia among the females of Rawalpindi-Islamabad. A survey was conducted to find out the prevalence of menorrhagia in females of Rawalpindi and Islamabad (N=6000). The data relevant to risk factors, causes and complications associated with menorrhagia was collected from patients (N= 103) visiting gynecology department of Benazir Bhutto Hospital Rawalpindi and Pakistan Institute of Medical Sciences, Islamabad. Prevalence of menorrhagia among the females of Rawalpindi and Islamabad was 4.8 percent. Major risk factors include fibroids that increased the AOR 21.992 times and hypothyroidism by AOR 6.893 times (P<0.05). Being married (AOR 1.938(.774-4.852) and multiparous condition (P<0.0000006) was also observed as a significant (P<0.05) risk factor. Present study found a strong association (P<0.05) between menorrhagia and its complications mainly anemia (84.4%), migraine (74.7%), anorexia (53.3%), insomnia (52.4%), fever (44.6%), digestive problems (21.3%) and pain (78.6%). Our study concludes that major risk factors associated with menorrhagia were fibroids and hypothyroidism. Anemia, migraine, anorexia, insomnia, fever, digestive problems and pain were major complications among menorrhagia patients.

**ALLOIMMUNIZATION IN MULTI-TRANSFUSED BETA THALASSEMIA MAJOR
PATIENTS VISITING SHAHEED ZULFIQAR ALI BHUTTO MEDICAL UNIVERSITY
(PIMS) HOSPITAL, ISLAMABAD**

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Beta thalassemia major is one of the major genetic disorders in Pakistan. Regular blood transfusion is the proposed management for the patients which is likely to be associated with development of red cell alloimmunization. The purpose of this study was to assess the rate and specificity of erythrocyte alloantibodies and to determine the variables affecting the extent of alloimmunization in addition to repeated blood transfusions. This prospective study included 475 repeatedly transfused thalassemia major patients. Alloantibodies were detected through 3-red cell antigen panel and their specificity was determined through 11-cell antigen panel. Alloantibodies were detected in 17 (16.2%) patients. 77 patients were found with 82 alloantibodies. Of these, 5

(6.5%) patients were found with double alloantibodies. Anti-D and Anti-E antibodies had highest incidence followed by Rh (c, C, e, Cw), Kell (K, Kpa, k), Kidd (Jka), Duffy (Fyb) and MNs (s) blood types. More alloantibodies were detected in males, splenectomized patients, in those who initiated their transfusions before 2 years of age, in patients receiving non leukoreduced blood and in B and O blood group patients. This frequency of alloantibodies production in multi-transfused patients is significant. The hemolytic nature of alloantibodies should be ascertained in alloantibody positive patients, and transfusion therapy should be managed accordingly.

CAUSES AND REMEDIES OF ARTHRITIS

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The purpose of this study is to conduct information about causes and remedies of arthritis, and to find the reason, why it is common in females? In order to check the arthritis disease, the month wise data was collected from different hospitals of Karachi during June 2016 to October 2016. Overall arthritic patients reported from different areas of Karachi. The total observed patients of arthritis were 266. Out of 266 patients, 144 patients were females and 122 patients were males. The age of arthritic patients were in between 45 to 75. Arthritis is not only bone disease it also affects the organs of the body like kidney, heart, liver, lungs. The main causes of Arthritis are obesity, old age, stress and lack of physical activities. Prevention should be taken to treat arthritis by injections, medicines and different therapies. Number of home remedies is implemented to treat arthritis, such as raw potato juice, banana, garlic, lime and different types of oils e.g. mustured oil, coconut oil and castor oil. Females are mostly affected by arthritis due to less amount of estrogen during and after menopause. Bones become thin and weak due to less production of estrogen hormone.

IDENTIFICATION OF MicroRNAs PROFILE RELATED TO PAKISTANI PCOS WOMEN

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Polycystic ovary syndrome (PCOS) is a heterogeneous disease of reproductive age. Small noncoding microRNAs (miRNAs) act as biomarker for PCOS patient stratification. An altered miRNA expression profile was observed in few previous studies. The aim of current study was to assess the miRNA expression profile and its risk factors specifically in Pakistani PCOS patients and healthy, regularly cycling matched controls. Experimental case-control study including 50 PCOS women and 50 healthy age and body mass index matched women. Anthropometric and other relevant clinical baseline measurements were obtained from each patient and control. Relative expression of miRNA levels were estimated using quantitative RT-PCR. PCOS women had decreased levels of miR-15, 195 and 457 compared with controls. Furthermore, miR-16 was differentially expressed within the PCOS group with high levels observed in the hyperandrogenic married group compared with the normo-androgenic PCOS patients. Distinction between PCOS and controls was made using miR-16 alone with an area under the curve of 0.91 or a combination

of four selected miRNAs (area under the curve, 0.93). Our study provides evidence that the miRNA expression profile altered in PCOS and indicates that specific miRNAs are associated with phenotypical traits of PCOS. An altered miRNA profile holds potentials for new methods of PCOS patient stratification and may contribute to and in part explain the heterogeneous nature found within PCOS women. All women of reproductive ages and most importantly young girls are at higher risk for different metabolic syndrome and Diabetes mellitus type 2; particularly obese young girls having hyperandrogenism are more susceptible to PCOS

ANTIBODIES AGAINST HEPATITIS C VIRUS 3a ENVELOPE GLYCOPROTEINS E1 AND E2 FOLLOWING CLONING, EXPRESSION AND PROTEIN PURIFICATION IN PAKISTAN

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Hepatitis C is a major global threat as more than 3% of the world population and 10% of the Pakistani population is chronically infected by Hepatitis C Virus (HCV). It may lead to liver cirrhosis, hepatocellular carcinoma (HCC) and eventually death. Current therapy has limited efficacy, more side effects and heavy cost. The present study is focused on the development of recombinant vaccine against HCV by utilizing envelope glycoproteins E1 and E2 of HCV to combat this disease. Blood samples were collected from the HCV 3a infected patients from local area. Total RNA was extracted through TRIzol reagent and cDNA was synthesized through random hexamers. Two sets of primers comprising of outer and inner primer were designed from Los Alamos. Nested polymerase Chain Reaction was carried out for E1 and E2 gene amplification. Sequencing confirmed the amplified genes E1 and E2 as HCV envelope glycoproteins. Cloning was performed by using PTG19 vector. Both E1 and E2 glycoproteins were expressed using PET21a expression vector in BL21 strain of *E. coli* as expression host. The E1 protein, expressed in soluble form, was purified through salting out procedure followed by anion exchange FPLC. The E2 protein, expressed in insoluble form, was purified by repeated sonications with 20mM Tris-Cl, 5mM NaCl and 0.01% Triton followed by denaturation and refolding through fractional dialysis with Urea. The purified proteins were injected into the rabbits and antibodies were allowed to raise which were confirmed through ELISA in rabbits as well as in human sera. It is concluded from the current study that purified HCV 3a envelope glycoproteins E1 and E2 have significant antigenic activity and can be used as recombinant vaccines against HCV infection.

HYPERTENSIVE EMERGENCIES

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High blood pressure is related to high occurrence of death. Globally 92 million population is affected. High blood pressure usually has no warning signs and the affected people do not feel sick. But in severe condition it leads to death. The increasing prevalence of hypertension in developing countries is of great concern. The prevalence of hypertensive emergencies in the present study is 84%. The purpose of this research is to identify the disease and its damages as well as its,

prevention and treatment. Study is conducted on medical wards of different hospitals of Karachi from July 2016 to October 2016. Study samples were consisting of 300 patients including males and females. Data was collected by using questionnaire (patient's age, disease, causes, health promoting behaviors and its treatment). Age of patients were above than 40 years. The most common forms of emergencies seen in this study were chronic kidney diseases, myocardial infarction, intracerebral hemorrhage, ischemic stroke, hypertensive retinopathy. Ratio of brain disease was highest among all. Most common causes seen in this study were, diet i.e., high salt and red meat intake, hereditary, sedentary life style, obesity, age factor, depression. Hypertensive damages mostly seen in females due to their obesity. With the help of different Health promoting behaviors this problem could be reduced. First line of treatment is to reduce stress, decrease level of sodium rich diet and red meat and exercise includes in routine on daily basis and most important is to educate patients regarding hypertension. Whereas, the second line of treatment includes antihypertensive therapy.

CO-RELATION OF ACUTE LEUKEMIA WITH CIGARETTE SMOKING

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Cigarette smoking is one of the most detrimental factors of leukemia. It is known that smoking increases the risk of leukemia almost 50 to 55%. This is commonly found in male as compared to female due to cultural norms. In the present study authors comprised a retrospective study in 50 patients with newly diagnosed acute lymphoid or acute myeloid leukemia in different hospitals of Karachi. In this study, 29 male and 21 female patients were our subjects which were approximately 58% and 42% respectively. Their ages were between 15 to 55 years. 60% patients were smoking at least one cigarette per day, we considered as smokers and remaining 40% are non-smokers. A positive but unpredictable or insignificant correlation (Pearson correlation) was observed among the cigarette smokers and leukemia patients by using the SPSS version 23. Acute lymphocytic leukemia is generally found in non-smokers. High rate of pulmonary infection also found in smokers because of the presence of many oncogenic materials are present. Firstly, these particles cause infection in pulmonary tract and then they absorb in blood stream and through blood they reach different organs of body and blood cells also where they cause malignancies. Other risk factors of acute leukemia is highly chemical exposures like benzene etc. and patients who have had certain type of chemotherapy or radiotherapy for the treatment of other type of cancer, that also increase the risk of leukemia, Age factor is also varies. Cigarette smoking has a harmful effect on the survival of leukemia especially acute myelogenous leukemia. Genetic variation is also play a significant role in the formation of leukemia.

PREVALANCE OF MALARIA IN DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

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Malaria is old as human civilization. Malaria is cause by *Plasmodium* which is a parasitic protozoan which passes some of its life time in mosquitoes and some part of its life in human being. According to Malarial Directorate of Pakistan one person per thousand populations has been

infected by malaria and this ratio is more in children. The present study was carry out in Swabi in three months that were from December 2015 to February 2016 in Tope, Ghanichatra and Gandaf to study prevalence of malaria and its form. The results of our study revealed that in 2013 in GhaniChatra a total of 1200 cases were registered in which 200 were positive for *Plasmodium falciparum*. Majority of them were children's. The results of our study revealed that in 2014 in Ghandaf a total of 1500 cases were registered in which 150 were positive for *Plasmodium falciparum*. Majority of them were children's. The results of our study revealed that in 2015 in Topia total of 1224 cases were registered in which 68 were positive for *Plasmodium falciparum*. Majority of them were children's. From the results of our present study it has been easily understand that malaria is a serious health problem in the rural areas of Swabi due to poverty, unawareness and contaminated ponds which provide a good reservoir for the breeding of larvae of mosquitoes which act as a vector for transmission of malaria. Therefore government and health department has to provide facilities to overcome this problem in Swabi.

PREVALENCE OF HEPATITIS B, C AND TUBERCULOSIS IN UNDER PRIVILEGED CHILDREN (ORPHANS) OF DISTRICT BAGH

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The hepatitis B, C and tuberculosis infections are among the major fatal diseases of the low socio economic communities around the world. Present work describes the incidence of these diseases in underprivileged children (orphans) of District Bagh Azad Jammu and Kashmir. After an extensive survey a total of 300 children (150 males 150 females) with an age limit of 4-17 years having informed consent were selected for the study. The blood samples were screened for Hepatitis C virus (HCV) antibody, Hepatitis B surface antigen (HBsAg) and anti-MTB antibodies by using ICT kit. Sputum microscopy for MTB was done in MTB antibody positive samples. The samples positive in screening were confirmed by Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). The evaluation of risk factors among the selected population regarding Hepatitis B, C and tuberculosis was performed with the help of questionnaire. Awareness among the selected area population was created by arranging seminar, distribution of pamphlets addressing the Hepatitis, Tuberculosis and associated diseases and their preventive measures. Out of total population (n = 300) no HBV positive case was found, whereas only one case (0.33 percent) was found positive for HCV in both ICT and ELISA, which further appeared negative in PCR. A total of seven (2.33 percent) cases were found positive for MTB in ICT and six (2.00 percent) in sputum microscopy and PCR. The evaluation of risk factors for hepatitis revealed that accidental prick (100 percent) along with tooth brush and comb sharing (98 percent) was the most predominant risk factor found in the studied population. It was observed that use of wood fuel (97 percent) was the most predominant risk factor of TB found in the studied population. Although the prevalence of Hepatitis B, C and TB among selected population was low but further studies are required to create awareness and keep the prevalence at same declining pace among peripheral communities of Azad Jammu and Kashmir.

SCREENING OF PESTE DES PETITS RUMINANTS VIRUS (PPRV) IN GOAT (*CAPRA HIRCUS LIN.*) POPULATION IN DISTRICT KHAIRPUR, PAKISTANABDUL KAREEM MAITLO¹, JAVED AHMED UJAN^{1,*} AND BADAR UDDIN A. MEMON²¹ Department of Zoology, Shah Abdul Latif University, Khairpur mirs, Sindh, Pakistan² Department of Microbiology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan

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Goats are the fastest growing ruminants in Pakistan and Pakistan is the third largest goat producing country in the world after China and India. Preference for goat meat is the major reason for its increased demand. There are 25 goat breeds in the country and two wild relatives such as Markhor and Ibex. At present, there are 53.8 million goats in Pakistan and their population is increasing at the rate of more than 3% per annum as per report of GOP, 2006). Punjab, Sindh, Balochistan and NWFP have 37%, 23%, 22% and 18% goat population, respectively. Peste des petits ruminants (PPR) is considered to be one of the main constraints to enhancing the productivity of goats and sheep in regions where it is present and becoming endemic and is a severe and highly infectious viral disease of small ruminants. The PPR virus (PPRV) belongs to the genus Morbillivirus in the family Paramyxoviridae. It is closely related to the rinderpest virus of bovines and buffaloes, distemper virus of dogs and other wild carnivores, human measles virus and Morbilliviruses of marine mammals. The purpose of this study was to determine the Screening of Peste Des Petits Ruminants Virus (PPRV) in Goat (*Capra Hircus Lin.*) population in district Khairpur, Pakistan. In present study we selected 290 goats which had sings of PPR clinically. Blood samples were taken then serum samples were achieved and analyzed by using a competitive ELISA kit according to the instructions of the manufacturer. Results of our investigation showed that of 92 clinically cases, 59 samples (64%) were positive and 33 samples (36%) were negative serologically. The conclusion from this study is that a PPR is probably more prevalent in the Khairpur district. Moreover, homologous PPR attenuated vaccine is highly recommended to be used to protect against virulent virus challenge in the country for control of PPR.

MOLECULAR AND PATHOGENICITY TYPING OF NEW LOCAL ISOLATES OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV) IN MALAYSIABASIT ZESHAN^{*1,2}, MUHAMMAD DANIAL HAFIZ MOHD SUADI NATA¹,
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Infectious bursal disease virus (IBDV) affects the bursa of fabricius of young chicken, resulting in morbidity, mortality and immunosuppression. Frequent outbreaks of IBDV has been observed in Malaysia due to emergence of variants strains, each with a different virulence. There is limited information on the currently prevalent field IBDV strains and their pathogenicity. Thus, the objective of current project was to study the pathogenicity and characterization of three local isolates of IBDV namely DV 53, DV 5/16, A1 isolated from Kelantan, Malaysia. Pathogenicity of the IBDV was done by inoculating the virus into allantoic cavity of ten days old embryo SPF eggs. The infected eggs were harvested and RT-PCR of VP2 gene of IBDV was carried out from infected

allantoic fluid for identification and characterization of virus followed by sequencing. Nephritis with presence of urate the kidney, petechial hemorrhage and enlarged of the liver, retard growth of the embryo was observed. RT-PCR showed expected band of 500bp in all three isolates while no band in the negative control. The sequencing followed by blast results showed that isolated have 98% similarity with submitted sequences in NCBI GenBank. The phylogenetic analysis showed that all three isolates fall under one cluster together with the Chinese isolates and were not matching with the Malaysian isolates. In conclusion, this study could give us preliminary data on the pathogenicity and PCR identification of new isolates of IBDV which could be useful for updating the current vaccines for the prevention and control of IBDV in Malaysia.

STUDIES ON THE ROLE OF HOUSE FLY AS A MECHANICAL VECTOR FOR NEWCASTLE DISEASE IN POULTRY

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Newcastle Disease Virus (NDV) is a highly infectious virus of domestic and wild birds and is excreted in the faecal material of infected birds. Commercial birds housed under environment controlled conditions or conventional housing, however, non-biting Muscid flies gained entry into the flock to feed on the bird's droppings having organic material and potentially contribute in infectious disease transmission. The house fly (*Musca domestica*) has been considered as a mechanical vector of numerous pathogens including NDV at commercial poultry farms. Therefore, in this study aerial net-captured houseflies were examined for the presence of NDV. Homogenate from house flies collected from infected poultry farms was first harvested in 11 days embryonated chicken eggs to propagate the NDV that was titrated using haemagglutination inhibition assay for quantification of NDV. The results of the current study showed three poultry farms positive for NDV. It was concluded that house fly serve as a vector for NDV and could be potentially responsible for transmission of NDV between the same poultry farm or to the closer farms.

EPIDEMIOLOGY AND ANTIMICROBIAL SENSITIVITY OF PATHOGENS ASSOCIATED WITH CAPRINE MASTITIS IN PAKISTAN

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Epidemiology and antimicrobial of mastitis causing pathogens in caprine was studied in the both rural and urban areas of district layyah, Pakistan. One Hundred and fifty milk samples, collected from 75 randomly selected lactating goats were subjected for the microbiological investigation. The detection of subclinical mastitis was done based on the SFMT (Surf Field

Mastitis Test). The findings of designed study show that *Staphylococcus aureus* was the most common prevalent pathogen, contributing for 52% isolates. *Streptococcus agalactiae* was second most commonly recovered isolates causing mastitis accounting for 19% of all isolates. Collectively, these two contagious mastitis associated pathogens accounted for almost 71%. Among the different nine commonly used antibiotics, gentamicin was found most effective against all mastitic bacterial isolates than others.

SERO-EPIDEMIOLOGY OF JOHNE'S DISEASE ALONG WITH ITS EFFECT ON SERUM BIOCHEMICAL PROFILE IN CATTLE IN DISTRICT D. I. KHAN, KHYBER PAKHTUNKHWA

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Johne's disease (JD) or paratuberculosis caused by *Mycobacterium avium* subsp. *Paratuberculosis* (MAP) is characterized by an incubation period of several years. It is a chronic non-treatable disease of animals causing chronic diarrhea, emaciated, edema of bottle jaw, serum protein leakage and ultimately lead to hypoproteinemia and albuminaemia cause economic losses to dairy and meat industries worldwide. In the present study indirect ELISA using commercial ELISA kit (*Mycobacterium Paratuberculosis* Antibody Test Kit, IDEXX, USA) were used for the screening of clinical and subclinical Johne's disease in cattle population in District DI Khan Khyber Pakhtunkhwa Pakistan. A total of 55 cattle were included in the study. Out of these 7 were positive and recorded the sero-prevalence (12.7%), the result were analysis by Pearson's Chi-Square and the serum protein analysis by t test by SPSS version 20. The data are highly significant in those cattle showing sign of chronic diarrhea, low BCS, old age >6 years, breed and management practices.

CONTROL OF MASTITIS THROUGH DRY COW THERAPY: A REVIEW

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Huge economic losses occur due to mastitis in the dairy industry all over the world. These losses can be reduced if mastitis is controlled by effective mastitis control programme. Among these programme dry cow therapy is very much effective to eliminate the existing intramammary infections and preventing new intramammary infections during the dry period and after calving. By adopting dry cow therapy through intramammary route at dry off or systemic dry cow therapy 2 weeks before parturition or combination of these two along with other proper mastitis control

managements, we can get rid of these injurious losses in dairy industry. In this review article, advantages of dry cow therapy, methods of dry cow therapy and practical aspects of dry cow therapy are discussed.

MORTALITY OCCURRED IN BROILER BIRDS DUE TO NEWCASTLE DISEASE IN DISTRICT KHAIRPUR MIRI'S, SINDH, PAKISTAN

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The present study was conducted to determine the mortality rate, occurred due to viral disease in broiler birds of Khairpur district. Four cities *i.e.* Kotdeji, Khura, Gambat, Kumb, and five poultry broiler farms were selected from each city during the study period 2014-2015. The study was conducted on the flocks history, clinical signs-symptoms and post mortem examination of the birds for confirmation of the infection. The data was collected randomly from each city. The results of the present study shows that, the total 674 number of birds affected due to Newcastle disease in all cities. The highest mortality 106 (1.81%) occurred in the Khura city as compared to other cities, while lowest number (0.92%) of birds observed in Gambat. It was concluded from present study that the Newcastle disease is viral disease of poultry which can affect vaccinated and non vaccinated flocks and caused heavy mortality among the broiler birds.

STUDY ON THE GROSS PATHOLOGY OF BROILER BIRDS AFFECTED WITH HYDROPERICARDIUM SYNDROME DISEASE IN BROILER POULTRY FARMS OF HYDERABAD AND ADJOINING CITIES, SINDH, PAKISTAN

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The Hydropericardium Syndrome popularly called the Litchi Heart disease, mostly of broiler chickens. It was first identified in broilers in Pakistan Angara village near Karachi in 1987. It spread rapidly in broiler producing areas; it affects mainly broilers and rear in broiler parents. Mortality may reach 60% but more typically 10-30%. During present study total 13599 number of affected broiler birds were examined for hydropericardium disease out of which 10939 number of pathological lesions were found in different organs of the body, *i.e.* liver, heart, kidneys, muscles, affected due to hydropericardium disease. The data was collected from the different commercial broiler farms of Hyderabad and its adjoining cities. For the confirmation of disease postmortem examination was conducted. Pathological changes *i.e.* discolouration, swelling, haemorrhages, necrosis, enlargement, varied in different visceral organs were also observed *i.e.* total number of liver 1622 (11.92%), hearts 3011 (22.14%), kidneys 1719 (12.64%), muscles 1349 (13.49%). For the prevention and control of hydropericardium disease the efficacy of vaccines can be improved, strict sanitation to reduce the losses from the disease in broiler flocks. However disease may become a serious problem in individual flocks or in selected geographic areas, vaccines have been shown to be effective against challenges with disease.

STUDIES OF GASTRIC GRANULOMA DUE TO ANISAKIS LARVAE IN THE CAT FISH, *ARIUS ARIUS* (HAMILTON, 1822) OFF KARACHI COASTABDUL SATTAR,¹ NASIRA KHATOON¹ AND ALY KHAN²¹*Department of Zoology, University of Karachi, Karachi-75270, Pakistan*²*Crop Diseases and Research Institute, University of Karachi, Karachi-75270, Pakistan*

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This investigation was undertaken to study the gastric granuloma in catfish, *Arius arius* (Hamilton, 1822) off the Karachi coast due to anisakis larvae. Anisakiasis is an increasing incidence in several countries of the world and has a zoonotic effect as well. This disease is adopted by eating raw or undercooked fish infected with anisakid larvae. The sampling was carried out in August, 2016. Infected fishes were purchased from different fish markets and brought to the parasitology laboratory for further detailed investigation by using standard parasitological procedures. The anisakid larvae were found in the gastric granuloma of host fish. Observations are based on the Bouin's fixed infected stomach of the fish. Standard procedures were used for the preparation of histological sections. The sections were stained with hematoxylin and eosin and then permanently mounted in Canada balsam. Granulomatous lesions and damage were observed in the gastric mucosa. Prominent spaces and holes were also observed in the gastric walls and produced granulomatous lesions. Numerous inflammatory cells were also observed around these lesions.

INVESTIGATION OF β - LACTAM ANTIBIOTIC RESIDUES IN MARKET (UNPROCESSED) MILK**Muhammad Husnain, Aneela Zameer Durrani* and Ikramulhaq***Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore*

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The present study was conducted to evaluate the extent of β - lactam antibiotic residues in unprocessed market milk during the year 2013. For the detection of antibiotic residues (Penicillin G, Ampicillin, and Amoxicillin) in milk, the samples were collected from local markets of district Lahore, in the Punjab province of Pakistan. A total of 120 samples (n= 30 zone-1 Mall Road; n=30 zone-2 Canal Bank Road; n=30 zone-3 Ferozpur Road; n=30 zone-4 Multan Road) were collected from raw (unprocessed) milk shops. These samples were screened for the presence of β -lactam antibiotic residues by using *Bacillus subtilis* (*B. subtilis*) Quantitative Field Disc Assay and for the quantification of these drugs in milk samples High Performance Liquid Chromatography (HPLC) method was used. The overall percentage of positive samples was 23% (28/120). Moreover, the distribution of positive samples among four zones was 17% (5/30), 10% (3/30), 30% (9/30) and 37% (11/30) in zone 1, 2, 3 and 4 respectively. In unprocessed market milk samples, the maximum zone of inhibition on *B. subtilis* growth was 14 mm while minimum zone observed was 3.5 mm. After quantification of these positive samples through (HPLC), ampicillin, amoxicillin and penicillin were present in 32 % (9/28), 85% (24/28) and 89% (25/28) of positive samples respectively. The results have also indicated that samples were not fit for human consumption having residues higher than (MRL's) standards of EU (4 μ g/L) and FDA (5 and 10 μ g/L), respectively. The percentage of the rejection out of positive samples was 100% (5/5), 100% (3/3), 67% (6/9) and 45% (5/11) in zone 1, 2, 3 and 4 respectively. Overall ignoring zone, percentage of the rejection out of positive samples was 68% (19/28). The percentage of the rejection out of total

milk samples collected was 17% (5/30), 10% (3/30), 20% (6/30) and 17% (5/30) in zone 1, 2, 3 and 4 respectively. Overall ignoring zone, percentage of the rejection out of total milk sample collected was 16% (19/120). The concentration of ampicillin in milk samples is approached significant ($P=0.06$) in zone 1 (9.9 ± 0.00 $\mu\text{g/L}$) than zone 2 (4.5 ± 0.00 $\mu\text{g/L}$), 3 (5.5 ± 0.15 $\mu\text{g/L}$) and 4 (7.6 ± 0.00 $\mu\text{g/L}$). However, concentration of amoxicillin and penicillin in milk samples was same among different zones.

4. MICROBIOLOGY

ROLE OF FLAGELLAR MOTILITY IN BIOFILM FORMATION OF PATHOGENIC BACTERIA

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Bacteria live either independently as planktonic cells or in organized surface associated colonies called as biofilms. Biofilms play an important role in increased pathogenesis of bacteria and it is assumed that motility is one of the contributing factor towards biofilm initiation. This study was planned to identify the role of flagella in biofilm formation by constructing flagellated (wild type) and physically disrupted variants (non-motile). Total 10 clinical bacterial strains were studied. On the basis of morphological variation and motility, only two highly resistant strains were characterized biochemically, physiologically and genetically. Biofilm formation capacity of strains was analysed using three methods including congo red assay, test tube assay and liquid-interface coverslip assay. Afterwards, flagellar disintegration was induced by blending and centrifugation for 5, 10 and 15 minutes. Our results showed these strains as *Bacillus cereus* and *Yersinia enterocolitica* identified by 16S rRNA sequencing. Both strains produced significant biofilm by all three above mentioned methods. A motility test of these blended variants showed partial leading to completely diminished motility with increased blending time. The significant loss in biofilm formation after 15 minutes of blending confirmed the important contribution of flagella to the initiation of biofilm formation. This biofilm defect observed in flagella paralysed/minus variants presumably may be due to defects in attachments to surface at early stages. This study indicated that flagellar motility is crucial initially for surface attachment and subsequently for biofilm formation.

INVESTIGATION OF β - LACTAM ANTIBIOTIC RESIDUES IN MARKET (UNPROCESSED) MILK

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β - Lactam antibiotic frequently used in veterinary for treatment of diseases, preventive and prophylactic purposes and to improve feed efficiency. Milk of antibiotic treated animals containing antibiotic residues for a few days after treatment. These residues may cause severe allergic reactions in sensitive consumers and contribute to antibiotic resistant infections in humans. The present study was conducted to evaluate the extent of β - lactam antibiotic residues in unprocessed market milk during the year 2013. For the detection of antibiotic residues (Penicillin G, Ampicillin, and Amoxicillin) in milk, the samples were collected from local markets of district Lahore, in the Punjab province of Pakistan. A total of 120 samples (n= 30 zone-1Mall Road; n=30 zone-2Canal Bank Road; n=30 zone-3Ferozpur Road; n=30 zone-4Multan Road) were collected from raw (unprocessed) milk shops. These samples were screened for the presence of β -lactam antibiotic residues by using *Bacillus subtilis* (*B. subtilis*) Quantitative Field Disc Assay and for the

quantification of these drugs in milk samples High Performance Liquid Chromatography (HPLC) method was used. Milk consumer health status survey was also conducted in the area of sampling. The overall percentage of positive samples was 23% (28/120). Moreover, the distribution of positive samples among four zones was 17% (5/30), 10% (3/30), 30% (9/30) and 37% (11/30) in zone 1, 2, 3 and 4 respectively. In unprocessed market milk samples, the maximum zone of inhibition on *B. subtilis* growth was 14 mm while minimum zone observed was 3.5 mm. After quantification of these positive samples through (HPLC), ampicillin, amoxicillin and penicillin were present in 32 % (9/28), 85% (24/28) and 89% (25/28) of positive samples respectively. The results have also indicated that samples were not fit for human consumption having residues higher than (MRL's) standards of EU (4µg/L) and FDA (5 and 10µg/L), respectively. The percentage of the rejection out of positive samples was 100% (5/5), 100% (3/3), 67% (6/9) and 45% (5/11) in zone 1, 2, 3 and 4 respectively. Overall ignoring zone, percentage of the rejection out of positive samples was 68% (19/28). The percentage of the rejection out of total milk samples collected was 17% (5/30), 10% (3/30), 20% (6/30) and 17% (5/30) in zone 1, 2, 3 and 4 respectively. Overall ignoring zone, percentage of the rejection out of total milk sample collected was 16% (19/120). The concentration of ampicillin in milk samples is approached significant ($P=0.06$) in zone 1 (9.9 ± 0.00 µg/L) than zone 2 (4.5 ± 0.00 µg/L), 3 (5.5 ± 0.15 µg/L) and 4 (7.6 ± 0.00 µg/L). However, concentration of amoxicillin and penicillin in milk samples was same among different zones. The survey showed that high potency antibiotics are used by consumers for different health problems. It is concluded that raw loose milk sold in the Lahore city had Penicillin and Amoxicillin residues. There is need for awareness of dairy animals, owners to use antibiotics in consultation to qualified veterinarian and give consideration to milk withdrawal period after administration of antibiotics to dairy animals. Most of the consumer have trend of using high potency antibiotics.

ROLE OF FUNGAL INDUCED STRESS IN BIOCHEMICAL AND PHYTOCHEMICAL PROFILING OF *PEGANUM HARMALA*

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Many biotic stresses are known to influence the production of secondary metabolites in plants including phenolic acids, biochemical and phytochemical compounds. The current study was designed to evaluate bioactive compounds under the influence of *Aspergillus niger* stress on seedlings of *Peganum harmala*. Various biologically active compounds were determined by using different techniques. The HPLC analysis detected significant amount of phenolic acids like gallic acid, caffeic acid and vanillic acid. Total protein content activity of proteases, α -amylases, catalases and peroxidases were observed to be accelerated under fungal stress at 4 and 5 days post inoculation (dpi) and then decreases at 6 and 7 dpi. The seedling extracts exhibited prominent antifungal and antimicrobial activity against certain bacterial strains including *Staphylococcus aureus* and *Bacillus subtilis*. The stronger antibacterial and antifungal activity was observed at 2-

4 dpi suggesting the change in bioactive profile due to biotic stress. Additionally, the extracts showed potential role as antioxidants in preventing H₂O₂ and UV light induced oxidative DNA damage. The results concluded that the potent antimicrobial potential of **Peganum harmala** makes it a good candidate to be used in natural therapies and medicine. In addition, antioxidants play key role in DNA repair mechanism and to combat against different oxidants.

ANTIBACTERIAL AND ANTIFUNGAL POTENTIAL OF EPIDERMAL MUCUS IN *CATLA CATLA*

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Fish lives in that environment which have pathogenic load. The purpose of present study is to measure the antibacterial and antifungal potential of epidermal mucus in *Catla catla* collected from the wild and controlled conditions. To achieve the goal of this study, at least 10 specimens of proposed fish was collected randomly from different sites of River Chenab. For comparison, fish specimens were also collected from controlled condition i.e. pond reservoir. Disc diffusion, Bradford method, Casein hydrolysis test and ion exchange chromatography by using DEAE-cellulose were used for detection of antimicrobial activity, protein concentration, protease activity and purification, respectively. Different body weights of *Catla catla* were also taken and variation in antimicrobial activity in accordance with weight was determined. Four types of bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus dysgalactiae*, and *Staphylococcus equisimilis*) and fungi (*Aspergillus terreus*, *Aspergillus niger*, *Pseudomonas putida* and *Trimates versicolor*) were used to check the potential of epidermal mucus of *Catla catla*. Mucus collected from wild habitat showed higher activity than that collected from control habitat while weight casted little or no affect on antimicrobial activity. Mucus of *Catla catla* showed satisfactory antimicrobial activity against all tested pathogens except *Trimates versicolor*. The maximum zone of inhibition was observed against *Staphylococcus aureus* (7.6 mm) while minimum for *Staphylococcus equisimilis* (3.2 mm) in case of bacteria and maximum diameter zone was exhibited by *Pseudomonas putida* (7 mm) in case of fungus. The mucus of *Catla catla* showed lesser antimicrobial activity when compared with standards, ciprofloxacin and ketoconazole. Evaluation of various immune parameters such as protein content and protease content was done and it was determined that level of protein was highest in mucus collected from wild site while protease activity was observed to be highest in mucus collected from control site. The results of Ion exchange chromatography revealed the fraction that showed highest specific activity in ion exchange chromatography from controlled mucus of *C. catla* measured was about 45.88 Umg⁻¹. Protein contents that this fraction showed was 1.59mgmL⁻¹ and the fractions that showed highest specific activity in ion exchange chromatography from wild mucus of *C. catla* were about 36.24Umg⁻¹ and protein contents that this fraction showed was remained 1.73mgmL⁻¹. The molecular weight of protease was measured by SDS-PAGE. In this study, Protease enzyme having molecular weight of 27KDa from epidermal mucus of *C. catla* was isolated.

BIOMINING APPROACHES AND ROLE OF MICROBIAL CONSORTIA IN BIOLEACHING OF COMMERCIAL METALS

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Bio-mining is the use of microorganisms for commercial extraction of lavish metals from ores and mine tailing with least effect on environment. Microbes play vital role in bioleaching procedures in commercial mining. The bacterial cells are used to detoxify/replace waste cyanide, marginal biomass and activated carbon. These methods are preferred over conventional techniques due to energy efficient, low cost, environment friendly and production of useful by-products. The microbe's (acidophilic, sulphobacillus, rhodococcus, ferrimicrobium & chemolithotrophic spp.) enhanced processes of copper and uranium bioleaching and biobenefication are in active use at industrial scale. About 20% of the world's copper is extracted using bioleaching. These procedures lead to oxidize insoluble metal sulphides to soluble sulphates. The isolation of thermophile's microbes for mineral biooxidation would lead to increase the commercial extraction of minerals at industrial scale. The conventional pyrometallurgical techniques have commercial concerns as they result in depletion of high grade ores and release of harmful gaseous emissions to environment. The main objective of bio-mining is decomposition of mineral profile and release of valuable metals followed by biooxidation and biotransformation. The microbe-assisted gold mining is known to double the yield of gold and needs to be fully explored using diverse array of microbes. Bioleaching is simple and low cost method for the developing countries with large ore deposits. About 30 strains of microbes have been discovered so far with potential impact on bioleaching. With advances in molecular genetics, physiology and microbial genomics, more promising approaches with increased bioactivities are possible. Further efforts are underway to culture exotic archaea followed by genome sequencing to enhance their potential as industrial tool for commercial bioleaching. The current review focuses the latest trends in bio-mining and implementation of modern genomic approaches to improve the microbial potential for commercial tasks.

ASSESSMENT OF SPRING WATER MICROBIOLOGY AND ROLE OF TYPHA ANGUSTATA AS BIOSORBENT

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Research studies on the purification of spring have mainly been carried out in developed countries and have focused on water purification systems using aluminium sulphate (a coagulant) and chlorine (a disinfectant). Such systems are expensive and not viable for rural communities due to abject poverty. There has been very little scientific research work into the use of plant extracts to purify spring water. This research was aimed at examining natural plant extract in order to develop

inexpensive ways for water treatment. Samples were collected from 25 selected locations. Microbial, physical and chemical parameters were analyzed. It was found that the majority of samples possessed high concentration of microbes. Out of 25 samples, 20 water samples showed maximum level of microbial contamination. In present research work, all water samples showed maximum number of colonies of microbes except sample No. 2, 7, 13, 23 and 24. Number of microbe colonies per Petri dish range from 85 to 279 with average of 136.4. The study involved creating an inventory of *Typha angustata* for water purification. A prioritization system was derived to select the most suitable extract, which took into account criteria such as availability, purification potential, yield and cost of extraction. Laboratory trials were undertaken on the most promising non-modified and modified extract of *Typha angustata* (leaves). Inhibition of microbes from water samples with the non-modified extract of plant ranges from 19% to 100% with average of 55.5% while inhibition of microbes from water samples with NaOH chemically modified extract of plant ranged from 2% to 98% with average of 46.4%. There is a need to carry out further studies, which include toxicity to guarantee the safety of using modified extract of *T. angustata* as a biosorbent in the purification of drinking water for human consumption.

MICROBES AS THERAPEUTIC AGENTS

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Microorganisms not only have importance in medical sciences as disease causing agents but have been extensively utilized as therapeutic agents. Besides being naturally helpful in preventing various diseases, they have also been genetically modified successfully for therapeutical purposes. Recombinant DNA technology has developed bioengineered microbes to bring improvements in human health. Various kinds of microbes including prokaryotic bacteria, eukaryotic fungi and viruses have been extensively modified to deliver therapeutically important proteins, drugs and act as a gene therapy vectors with high degree of site specifications. Among various microbial strains *E. coli*, *S. cerevisie* and *P. pastoris* have been found to show high potency for producing therapeutic proteins which include various hormones, enzymes, blood proteins, antibodies and antimicrobials etc. However owing to the eukaryotic nature, fungal strains *S. cerevisie* and *P. pastoris* have been preferred over bacterial strains for human therapeutic protein production. Besides *E. coli* strains, many non-pathogenic and pathogenic bacterial strains have also being genetically modified such as a group of Lactic acid bacteria modified successfully for treating antibiotic resistant diseases and found to bring immunization thus representing themselves as an excellent candidate vaccine as compared to a virulent pathogenic strains. Non-pathogenic modified strains have been found to show more efficiency in anticancer therapies. In addition to that, viral vectors through gene replacement therapy have brought remedy to various diseases by providing solution at genetic level, enabling human body to produce proteins naturally. So microbes can be extensively utilized as great source of therapeutic agents.

**ANALYSIS OF BACTERIAL LOAD IN PRE AND POST FILTERED DRINKING WATER
AT THE EDUCATIONAL INSTITUTIONS OF RABWAH,
DISTRICT CHNIOT, PAKISTAN**

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Water-borne diseases due to bacterial contamination is one of the major cause of mortality in the children of developing countries. The main objective of the study was to evaluate the bacterial load in drinking water of educational institutes at Rabwah town, District Chiniot, Pakistan and to recommend best practices to provide clean drinking water. Samples were collected on a monthly basis for continuous 10 months (March 2016 - December 2016) from 15 educational institutes before and after the filtration to check the effectiveness. Each sample was processed for the analysis of bacterial load and also the detection of *E.coli* through the plate detection method. Confirmatory biochemical tests were also performed for the detection of *E.coli*. Results showed that percentage of pre-filtered samples of all the institutions is highly contaminated as compared to the post filtered. 2 institutions out of 15 showed the contamination of *E.coli* in pre filtered sample and only one institution in post filtered. Because of improved filtration methods the reduction in bacterial load was detected. Cleaning of water tanks/reservoirs showed the bacterial reduction to 16.6%, cleaning of pipes showed 24.3% and changing frequency of filters according to the time represent 21.9% reduction in bacterial load. The study showed that improved filtration methods could decrease the bacterial load in drinking water and mainly the pipes are the major reservoir of bacteria. By proper cleaning of pipes, bacterial load could be reduced to the higher extent than other filtration methods.

**COMPARATIVE STUDY OF BACTERIOICIN AND ANTIBIOTICS EMPLOYED TO
TREAT UPPER RESPIRATORY TRACT INFECTIONS IN LOCAL
POPULATION OF DISTRICT SARGODHA**

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Resistance in bacteria is increasing worldwide. Commonly acquired infections like respiratory infections caused by bacteria are usually found resistant. The current project was aimed to isolate antibiotic resistant bacteria causing upper respiratory tract infections (URTI) and to compare the efficacy of bacteriocin in comparison with commonly used antibiotics. Three bacterial strains (*Moraxella catarrhalis*, *Neisseria meningitidis* and *Alcaligenes sargodhensis*) were isolated from throat swabs of (391/439) patients suffering from URTI. Bacteriocin was obtained from *L. acidophilus* following standard available procedures. Molecular characterization of bacteriocin was also performed using SDS-PAGE. For comparison of anti pathogenic activity of bacteriocin and commercially available antibiotics, different concentrations of antibiotics were employed against pure bacteriocin. The results of study indicated that normal micro flora of throat that was considered safe is now supporting the pathogenic community, to cause infection *Alcaligenes sargodhensis* was found to be novel strain in this study based on results of ribotyping and Phylogenetic relationship with *Alcaligenes* family. All strains (*Moraxella catarrhalis*, *Neisseria*

meningitidis and *Alcaligenes sargodhrensensis*) were found resistant to antibiotics (moxifloxacin, ciprofloxacin, levofloxacin, amoxicillin and erythromycin) at more and less extent. Activity of bacteriocin was found comparable with antibiotics to control the antibiotics resistant bacterial strains. The present study suggests bacteriocin as safe conjugate of bacteriocin to deal with the antibiotic resistant infections particularly community acquired infections as it not only has antibacterial activity comparable with antibiotics but also in strengthens the immune system against bacterial attack.

ISOLATION AND IDENTIFICATION OF FUNGAL PATHOGENS FROM *CLARIAS BATRACHUS* (HYBRID) CULTURED UNDER SEMI-INTENSIVE CULTURE SYSTEM

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The aim of this study was to investigate fungal infections in a commercial freshwater fish, *Clarias batrachus* (hybrid). The fish samples were obtained from ponds of University of Veterinary & Animal Sciences, Lahore (Ravi Campus). Eight samples of this species were studied for the presence of fungal infections. Three fungal species namely *Aspergillus penicilloides*, *Aspergillus* sp. and *Aspergillus niger* were isolated from the fish. These fungal species have been found to cause serious diseases in commercial freshwater fishes. Fungal pathogens diminish the value of both fish and its flesh. Bad water quality enhances the fungal growth which affects the whole life stages of fishes. This study showed that most of the fungi isolated from fishes are considered as normal mycoflora yet many fungi can cause natural infections in ponds and aquaria. Attention must be paid to fish health management practices in rearing facilities.

FREQUENCY AND DRUG RESISTANCE OF *PROTEUS MIRABILIS* IN URINE OF CATHETERIZED PATIENTS.

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Catheter-associated urinary tract infections remain the most common nosocomial infection. The aim of present study was to determine the frequency of *Proteus mirabilis* infection and its antibiotic resistance pattern in the urine of catheterized patients. A total of 100 urine samples of catheterized patients were collected from a hospital of Lahore. Samples were cultured on blood, CLED and MacConkey agar plates for isolation of bacteria. Bacteria were biochemically identified. Drug resistance pattern of *Proteus mirabilis* against different antibiotics was tested by Kirby Bauer disc diffusion method. Out of 100 urine samples, 13 showed positive growth for *Proteus mirabilis*. Among 13 isolates, 84.61% were resistant to Nalidixic acid, 61.53% to Amoxicillin, 53.84% to Ampicillin, 46.15% to Amikacin, 38.46% to Ciprofloxacin and 30.76% were resistant to Levofloxacin while 0% *Proteus mirabilis* isolates showed resistance to Imipenem. In conclusion

Imipenem was the most effective antibiotic for the treatment of UTI caused by *Proteus mirabilis* in catheterized patients.

ISOLATION AND IDENTIFICATION OF FUNGAL PATHOGENS FROM *CLARIAS BATRACHUS* (HYBRID) CULTURED UNDER SEMI-INTENSIVE CULTURE SYSTEM

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The present study was conducted to evaluate the impact of industrial and anthropogenic discharges on water quality of the river Ravi, Pakistan. For this purpose, six sampling sites at variable distances along the river stretch were chosen to analyze the water quality. The sampling sites were; Ravi Siphon (A), Shahdara (B), Shah Pur (C), Sundar (D), Manga Mandi (E) and Balloki Headworks (F). The result showed that at point A, there was low contamination due to no point source of pollution. Alongside M.R link and B.R.B link canals dilutes the water of the river. The point B seemed as the starting point of pollution due to Mehmood Boti Drain, so some water quality parameters were above the permissible values. At point C, load of various pollutants was found due to addition of Multan Road Outfall and Gulshan-e-Ravi Outfall. At sites D and E, there was great pressure of pollution by anthropogenic waste and industrial discharges. Hudiara and Deg Drains added to the river at these sites and badly affect the chemistry of the River water. The present study suggests the dumping of only treated industrial effluents into the main aquatic bodies.

PHYSICO-CHEMICAL STATUS AND COLIFORM ANALYSIS OF WATER OF VILLAGE KHILLAH MUZAFFARABAD AJ&K

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Groundwater is generally considered a "safe source" of drinking water because it is abstracted with low microbial load with little need for treatment before drinking. However, groundwater resources are commonly vulnerable to pollution, which may degrade their quality. An assessment of microbial and physicochemical qualities of springs, stream, coal mine and Tap water in village Khillah (near airport) Muzaffarabad was conducted with aim to determine the levels of physicochemical parameters (temperature, pH, turbidity, alkalinity, Ca⁺², Cl⁻¹, Mg⁺², S⁻², TDS, TSS, TOS, TIS) and bacteriological (*E. coli*) contaminants in drinking water using standard method. All the water quality parameters were within standards set by WHO, only coliforms (*E.coli*) were present in high concentrations during rainy season. One-way ANOVA shows that all other parameters of different samples are same, as their P values are greater than 0.05 except temperature, alkalinity Ca⁺² Mg⁺² and S⁻² which differs significantly.

**ISOLATION AND CHARACTERIZATION OF BACTERIAL POPULATION
FROM POST-SURGICAL WOUNDS OF PATIENTS FROM
AYUB MEDICAL INSTITUTE, ABBOTTABAD**

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Post-surgical wound infection becomes more common and severe problem of today. This study was design to isolate and characterize the most frequent pathogenic bacteria in post-surgical wound. A total of 64 pus sample were taken from patient of Ayub Medical Institute Abbottabad. The culture media nutrient Agar, Potato dextrose Agar and Malt extract were used for the isolation of bacteria species from contaminated wounds. Identification test carried out according to standard procedure in order to identify the bacterial species. In the present study nine species were isolated. *Staphylococcus aureus*, (75%) was the commonest specie followed by *Staphylococcus saprophyticus* (70.31%), *E. coli* (70%), *Enterococcus faecalis* (56.25%), *Pseudomonas aeruginosa* (50%), *Klebsiella pneumonia* (42.18%), *Proteus mirabilis* (34.37%), *Enterobacter spp* (23.43%) and *Streptococcus spp* (23.43%).

**IDENTIFICATION OF BACTERIAL PATHOGENS FROM EYE
INFECTIONS AND THEIR CONTROL THROUGH CHEMOTHERAPY,
PHYTOTHERAPY AND APITHERAPY**

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The present study was conducted to isolate and characterize pathogenic bacteria from eye infections. The samples were taken from corneal eye infections. The collection of samples was done from "Layton Rehmat Eye Hospital, Township Lahore". Isolated bacterial pathogens were identified on the basis of morphological and various biochemical tests. Identification of Pathogenic bacterial isolates was confirmed by growing on selective medium. Antibacterial activity of different compounds was observed against the pathogenic bacterial isolates. Minimum Inhibitory Concentration (MIC) test of different honeys was performed against the isolated bacterial pathogens. The antibacterial activity of leaf extract of *Azadiracta indica* (Neem Plant) was also checked against the pathogenic bacterial isolates. The bacterial pathogens isolated from eye infections were identified as *Staphylococcus sp.*, *Streptococcus sp.*, *Neisseria sp.*, and *Bacillus sp.* These bacteria commonly caused corneal infections. All the pathogenic bacterial isolates showed sensitivity against all the compounds except *Streptococcus sp.* (BS1) and it showed resistance only against 3-Nitro-aniline (SAHC-11). Bacterial isolate *Staphylococcus sp.* (BS4c) showed maximum sensitivity against methyl-amine (SAH-5) with zone of inhibition of about 16mm in diameter while the bacterial isolate *Staphylococcus sp.* (BS6) showed minimum sensitivity against Propyl-diamine (SAH-16) and Ethylamine cinnamaldehyde derivative (SAHC-7) compounds with 1mm diameter

of zone of inhibition under optimum growth conditions *i.e.*, optimum pH and temperature. Antibacterial activity of leaf extract of *Azadiracta indica* (Neem Plant) was also checked. Methanol is used as standard against which all the pathogenic bacterial isolates showed resistance. The minimum sensitivity showed by *Staphylococcus sp.* (BS4C) with zone of inhibition of about 2mm in diameter while the maximum sensitivity showed by *Staphylococcus sp.* (BS6) with zone of inhibition of 8mm. MIC test of four different honeys was also performed. Different honeys showed different results. For all the isolated bacterial pathogens, Pak honey inhibited the growth at 1ml concentration, Local honey at 3ml concentration, Marhaba's honey at 4ml concentration, and Young's honey inhibited the growth at the concentration of 5ml.

**MOLECULAR CHARACTERIZATION OF MULTI DRUG RESISTANT
ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS* FROM PATIENTS IN
AZAD JAMMU AND KASHMIR**

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The burden of disease and death caused by tuberculosis (TB) makes it one of the most important and dangerous diseases in the history of mankind. Globally, Pakistan, with population of around 180 million, is at 5th spot in the list of countries with the highest number of TB patients in the world. Global TB control is further challenged by drug-resistant TB. The present study was conducted to estimate Multi Drug-Resistant (MDR) tuberculosis, to analyse mutations responsible for the drug-resistant tuberculosis and to control the spread, better understanding of *M. tuberculosis* in Azad Jammu and Kashmir (AJK). A total of 463 suspected patients of AJK having mean age of 41.54 were selected for this study from January 2014 to December 2015. These suspected patients were examined and their samples were tested through Gene Xpert to find out Rifampicin (Rif) resistance. A total of 3.8 percent Rif resistant cases were detected in Abbas Institute of Medical Science AJK. Drug resistant genes analysis was done in National Reference Lab Islamabad. 37 TB patients were further processed through Line Probe Assay (LPA) to analyse common mutations responsible against first line drugs. Genes associated with drug resistance like *katG* and *inhA* for Isoniazid (INH), *rpoB* for RIF resistant isolates were checked for mutation using PCR amplification and LPA. It was observed that out of 37 MDR patients 25 patients (67.5 percent) had mutations in *rpoB* gene. Observed mutations were at codon 531 (72 percent) and 526 (16 percent) while 27 (72.9 percent) patients were resistant to INH. 22 (59.4 percent) patients had *katG* mutation at codon 315. The C to T transition at point -15 for *inhA* promoter region was also observed in 2 (0.054 percent) patients responsible to resist INH. Mutations in INH, *rpoB* and *katG* mutations were found in drug resistant isolates of MTB at a frequency of 72.9%, 67.5% and 59.4% respectively. Our findings suggest the large scale awareness and timely diagnosis of TB to ensure the timely treatment and in turn the life safety of affected population.

ASSESSMENT OF ANTIBIOTICS SUSCEPTIBILITY PROFILES OF *KLEBSIELLA* ISOLATES FROM HOSPITALIZED PATIENTS IN DISTRICT HARIPURSUMBAL BANO¹, ZAHID MAQBOOL¹, MUHAMMAD AKMAL SHAH¹ AND MOHAMMAD SALIM²¹Department of Zoology, Government Postgraduate College Haripur, Khyber Pakhtunkhwa,²Department of Forestry and Wildlife Management, University of Haripur, Khyber Pakhtunkhwa,

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Klebsiella is among the five gram negative pathogens most commonly encountered in hospital acquired infections. The pathogenicity caused by genus *Klebsiella* is due to its ability of showing resistance against antibiotics and production of toxin that causes different infections in host body. This study was conducted on six *Klebsiella* strains for measuring the antibiotic susceptibility by disc diffusion method. Out of eleven antibiotics ten were showed 100% effectiveness against six *Klebsiella* strains which were isolated from urine, blood and pus swab of different hospitalized patients. Further 83.33% strains exhibited susceptibility to Ceftazidime-pentahydrate while 16.6% strains were found resistant against Ceftazidime-pentahydrate. It was concluded that *Klebsiella* strains showed maximum (100%) susceptibility to Ceftriaxone-zinc, Cefepime hydrochloric acid, Cefotaxime sodium, Ceftriaxone iron, Cefazoline sodium, Ceftriaxone, Ciprofloxacin, Levofloxacin, Azithromycin, Gemifloxacin, while maximum resistance (16.6%) was only found to be against Ceftazidime pentahydrate.

BASELINE STUDY OF MICROBIAL CONTAMINATION IN DIET AND HOUSING FACILITIES OF *PANTHERA LEO* HOUSED AT DIFFERENT CAPTIVE CONDITIONS OF PUNJABALI RAZA,^{1*} ROHEELA YASMEEN,² BUSHRA NISAR KHAN³ AND RIDA AHMAD⁴^{1,2} Lahore Garrison University, Lahore, Pakistan.³Centre for the Undergraduate Studies University of the Punjab, Lahore, Pakistan.⁴Department of Zoology University of the Punjab, Lahore, Pakistan.

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A study was planned to access the microbial contamination in samples of beef, water, soil, and fecal collected from cages of African lions housed at Lahore Zoo, Zoo Safari, Bhawalpur Zoo and Islamabad Zoo by identifying and quantifying the microorganisms. The aim of study was to determine the maintenance conditions of lions in different captive sites and to provide the baseline data of microbial fauna in lion's diet and housing facilities. The results indicated that Gram-negative bacteria *Escherichia coli*, *Enterobacter species*, *Proteus species*, *Citrobacter species* and *Acinetobacter species* were predominated in samples collected from Lahore Zoo, Safari Zoo, and Bhawalpur Zoo. The fecal and beef samples of these localities were heavily contaminated by *Escherichia coli*, *Citrobacter*, and *proteu* while in soil and water the dominated bacterial group was Gram-negative bacilli (*Pseudomonas sp.*). Among selected sites the samples of water, soil, beef and fecal collected from Islamabad Zoo has shown lower level of bacterial contamination without any pathogenic species. It is also evident from results that food and maintenance conditions of Islamabad Zoo are better suited for African lion then Lahore Zoo, Zoo Safari and Bhawalpur Zoo.

ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIAL SPECIES FROM FOOD SAMPLES, COLLECTED FROM DIFFERENT LOCALITIES OF LAHORE

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Food-borne bacteria have been the most concern in public health and food safety. These bacteria lead to the nosocomial and many other infections. Foodstuff contamination may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of foods. The main objective of the present microbial study was to isolate and identify the pathogenic microorganisms from food samples (Dahi bhalay, Fruit chaat, Fruit juices, Salad, vegetables, and meat) to create public awareness about the health hazards resulting from these pathogenic microorganisms. Food samples are contaminated with pathogens that can cause food poisoning. *Escherichia coli* O157:H7 is one of them that cause several diseases in humans and animals. A more than 100 samples of each food items were collected from different localities of Lahore. All the samples were first cultured on nutrient broth and nutrient agar, which ensured the presence of certain pathogenic microorganisms. For further confirmation and identification, the culture from the nutrient agar was streaked on different selective media and the presence of pathogenic bacteria like *Staphylococcus sp*, *Salmonella sp* and *E. coli* O157 was confirmed. Presence of pathogenic *E. coli* O157:H7 was confirmed by using kit containing antiserum against bacterial specie. Results indicate that samples are much contaminated. The external surfaces of exposed meats that are present on different shops are responsible for the transmission of the infectious diseases by pathogenic bacteria. The results shows that meat products may act as an important vehicle of transmission for well-established pathogens and this is due to unhygienic conditions of the environment and can cause a danger to the public health when they are touched by these contaminated meats. So it is necessary to do proper washing and avoid precooked meat, and open food, for eating purposes.

BIOSYNTHESIS OF XYLANASE FROM MIXED FUNGAL CULTURE BY USING SUBMERGED FERMENTATION

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Xylanase has wide spectrum of biotechnological applications in different industries such as jute mill, animal feed, food, drinks and bleaching of cellulosic pulp. The present study concerns with isolation, screening and optimization of cultural conditions for xylanase production from mixed fungal culture. Different fungal strains were isolated from different soil sample and screened qualitatively on the basis of xylan hydrolysis zone. Quantitative screening of 46 compatible mixed culture was carried in submerged fermentation. The mixed culture No.20 showed highest xylanolytic potential was identified. It consists of *Aspergillus niger* and *Aspergillus flavus*. Six

different media were evaluated for the production of xylanase by mixed fungal culture. The M5 medium containing (g/l): Wheat bran 4, Yeast extract 5.0, Na₂HPO₄·2H₂O 10.0, KCl 0.5 and MgSO₄·7H₂O 0.15 gave maximum xylanase production. The other physical and nutritional factors including incubation time, temperature, pH, size of inoculum, volume of medium, carbon and nitrogen sources were also optimized. The maximum production of xylanase was obtained after 96 h at 30°C, pH 5, inoculum size 4%, and 50ml fermentation medium. Xylose (1%) and NaNO₃ (0.5%) were found to be best carbon and nitrogen source, respectively. Influence of different metal ion and surfactant was also noted. Optimal production of xylanase (153U/ml) was obtained in the presence of 0.2% ZnSO₄ and 0.1% Tween 80.

5. MOLECULAR BIOLOGY

THE MITOCHONDRIAL GENOMICS AND PHYLOGENETIC ANALYSIS OF SNOW TROUT (*SCHIZOTHORAX PLAGIOSTOMUS*) FROM RIVER PANJKORA, KHYBER PAKHTUNKHAWA, PAKISTAN

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The complete mitochondrial genome is of prime importance for better understanding of genomic characteristics and phylogenetics relationships among related species. The present study determined the complete mitochondrial genome DNA sequence of Snow trout (*Schizothorax plagiostomus*), which was 61563 bp in length with 37 genes, 13 protein coding genes, 2 ribosomal RNA (rRNA) genes, 22 transfer (tRNA) genes and one putative control region (CR). Of the 37 genes, twenty eight genes were encoded on heavy strand (H), while remaining nine genes were located on light strand (L). The genomic arrangement and organization of the *Schizothorax plagiostomus* was similar to other cyprinid fishes. The nucleotide compositions were, A 30.07%, G 16.78%, C 28.2% and T 25.16%, while A+T content was 55.3% and G+C content was 47.7% respectively. All the protein coding genes had ATG as start codon except for cytochrome b gene with GTG start codon. In *Schizothorax plagiostomus*, the *16SrRNA* genes lie between the *tRNA-Val* and *tRNA-Leu* (UUR) genes, while the *12SrRNA* lies between the *tRNA-Phe* and *tRNA-Val*. The control regions of *Schizothorax plagiostomus* were located between the *tRNA-Pro* and *tRNA-Phe* genes (936 bp) with a putative termination-associated sequence (TAS). Several conserved sequence blocks (CSB) were also identified within D-loop sequences. The phylogenetic analysis based on complete mitochondrial genome sequence showed molecular genetic relationship of *Schizothorax plagiostomus* with 24 closely related species (mostly cyprinid) and his mitogenome sequence would be useful to know the phylogenetic status of the *Schizothorax plagiostomus*.

SECONDARY STRUCTURES IN TRANSLATION INITIATION REGION DETERMINES CRY2 EXPRESSION IN *BACILLUS* HOST

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The major cause of destruction of agricultural crops is insect pests. Pesticides play significant role in improving agricultural production, but their haphazard use causes harmful effects on the non-target organisms, human health and ultimately pollute the environment. *Bacillus thuringiensis* produce insecticidal crystals protein during sporulation phase and acts as safer alternative to synthetic counterparts. Cry2A acts as bioinsecticides towards agricultural and public health significant order Lepidoptera and Diptera respectively. The gene *cry2Aa* are positioned at

third position (Orf3) in operon. It needs accessory proteins for crystal formation and high yield. The 29-kDa (Orf2) protein in the *cry2Ac11* operon increases yield and assists in crystallization of each molecule after synthesis. Various mutations in spacer region between RBS and initiation codon of *cry2Ac11* were introduced in order to determine the role of secondary structure in translation initiation region. Mutants were expressed in acrySTALLIFEROUS *Bt* strain. RBS-ATG mutagenesis was analyzed by SDS-PAGE. Expression level of Cry2Ac has been affected by alteration in number of nucleotides in this region. Thus, it was concluded that sequence of nucleotides between RBS and ATG is critical for expression in *cry2Ac11* gene. As expression of Cry toxin gene size in plants can be considered as limiting factor, so this study going to solve this problem.

RNA INTERFERENCE: A POTENTIAL MOLECULAR TOOL TO CONTROL MOSQUITO AND MOSQUITO BORNE DISEASES

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The most deadly animal in the world is mosquito. It might seem impossible that something so miniscule can kill so many people but it's true. According to World Health Organization mosquito bites result in more than 1 million deaths per year and 500 million cases occur every year. There are more than 3500 species of mosquitoes found in over 100 countries and every region apart from Antarctica. Mosquitoes responsible for transmission of various vector borne diseases e.g. dengue fever, malaria, yellow fever, zika fever, filariasis in humans as they are the reservoir host of many pathogens. During their blood feeding they transmit pathogens and number of proteins regulate this process of feeding. This blood sucking become successful through the secretion of anticoagulants, antiplatelets, and vasodilatory substances in their saliva so that they can easily get their meal. An important tool used to control these emerging diseases is RNAi. This technique is used to determine and silence the gene responsible for propagation of pathogens within vector and transmission to the host. By using this technique scientists silenced several genes involved in pathogen transmission. In this technique dsRNA, siRNA (short interfering RNA), Dicer, and RISC (RNA induced silencing complex) are used. dsRNA is injected into vector which silence the target gene expression. Dicer enzyme convert the dsRNA into siRNA and also load the siRNA into silencing complex RISC. It target the mRNA of target gene and cause degeneration of target mRNA. Phenotypic expression of vector show that gene is silenced. Several genes involved in the propagation of pathogen within vector for transmission to the host can be silenced by this technique. And ultimately several pathogens e.g. bacteria, viruses, protozoa can be inhibited to transmit to the host and disease can be avoided. Hence several diseases of the animals and humans transmitted due to these vectors can be avoided.

**MOLECULAR DETECTION OF *BABESIA MICROTI* IN DOGS
FROM PUNJAB (PAKISTAN)**

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Babesia microti is an intraerythrocytic protozoan that mainly infects red blood cells and causes babesiosis. Its frequent hosts are rodents, ticks and humans. 150 Dog's blood samples were collected from three different districts in Punjab including Multan (N = 49), Islamabad/Rawalpindi (N = 49) and Lahore (N = 52). Data on the epidemiological characters (including age, gender, breed, body temperature, deworming, vaccination, mucus membrane status, hydration status, presence of Hematuria and tick infestation) was collected through questionnaire. Polymerase chain reaction (PCR) amplified a 238 base pair amplicon specific for 18S rRNA gene of *Babesia microti* in two (1.3%) out of 150 dogs blood samples. Among epidemiological factors, high body temperature ($P < 0.05$) and pale mucous membrane ($P < 0.05$) were the only parameters associated with the presence of *Babesia microti* and infected dogs. Various hematological parameters were also determined and compared between *Babesia microti* positive and negative blood samples. Granulocytes were increased ($P < 0.01$) and mean corpuscular hemoglobin concentration ($P < 0.001$) were significantly decreased in *Babesia microti* positive samples as compared to parasite negative samples. As babesiosis is newly emerging disease in dogs, we recommend this PCR protocol for the diagnosis of *Babesia microti* in dogs.

**CLONING AND SEQUENCING OF *M. tb* Gene TrxC, A STEP TOWARDS
DEVELOPMENT OF SUBUNIT BASED DNA VACCINE AGAINST TUBERCULOSIS**

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Tuberculosis (TB) is an air-borne bacterial infection that affect lymph nodes, joints, bones, brain and most often lungs. DNA vaccine is a novel technique used to efficiently stimulate humoral and cellular mediated responses against infections. DNA vaccines have entered into a variety of human clinical trials against Tuberculosis. The present study aimed to design sub-unit based DNA vaccine against TB by using *M. tb* gene TrxC. TB. For this purpose TrxC gene was amplified using *M.tb* DNA with PCR. The amplified product was ligated into pTZR vector. The Construct TrxC-pTZR was then transformed into α -DH5 *E. coli* competent cells. The transformed colonies were grown on agar plates and then onto broth media, these were further processed for plasmid extraction using EZ-10 Spin Column Endotoxin Free Plasmid Preps Kit. The DNA was confirmed by restriction digestion with restriction enzymes BglIII and XbaI to get its fragments. The size of DNA fragments was observed by running it on 1% agarose gel with 1KB ladder. Further work is needed to evaluate the efficiency of this construct against TB.

ANALYSIS OF THE POPULATIONS OF THE *CIRRHINUS MRIGALA* AT DIFFERENT GEOGRAPHICAL SITES AT INDUS RIVERINE SYSTEM OF PAKISTAN BY MITOCHONDRIAL ATPASE 6/8 GENES

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This study was mapping the genetic diversity of *Cirrhinus mrigala* by using molecular markers for the ATPase 6/8 gene region. Samples of the target fish species were collected from the different sites at the Indus riverine system of the Pakistan i.e. river Chenab, river Jhelum, river Ravi and river Indus. Phenol Chloroform Method of Genomic DNA extraction was used to extract whole blood DNA. The PCR product was confirm on 1.2% agarose gel. After the visualizing of quality of band confirmation the samples were sent to be sequencing.

PHYLOGENETIC RECONSTRUCTION OF *ROPALIDA BREVITA* (VESPIDAE: POLISTINAE) USING 12S rRNA AND CYTOCHROME OXIDASE 1(CO1) GENE

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A total of 67 samples of *Ropalida brevita* were collected from District Abbottabad, Pakistan during 2011 to 2013, and taxonomic key was developed to the specie level. Which were belonging to subfamily of Polistinae. Phylogenetic tree were reconstructed using 12S rRNA (12S) gene showing homology of 73% compared with gene bank data while on the basis of cytochrome oxidase 1 (CO1) gene 93% homology were recorded. It was concluded that CO1 gene is more reliable than 12S rRNA gene for confirmation of *R. brevita* position in the Vespidae family and evolved in Southeast Asia and dispersal to the New World.

CHARACTERIZATION OF Cry2Ac7 A NOVEL CRY PROTEIN TO CONTROL *ANTICARSIA GEMMATALIS*

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Anticarsia gemmatalis (velvetbean caterpillar), a lepidopteran insect, is a major pest of soybean and other crops like pea, wheat and rice and cause serious damage by defoliating the

plants. *Bacillus thuringiensis* crystal proteins have insecticidal activities against many notorious pests of economically important crops. Cry2Ac7 is a short type of Cry proteins. *E.coli* BL21 CodonPlus (DE3)-RIL cells were transformed with cry2Ac7pET28 recombinant plasmid and protein was overexpressed using 0.1 mM IPTG for 6 hours in LB medium at 37 °C. Protein inclusion bodies with His-tag denatured in urea was on-column refolded and purified using Ni-NTA resin and decreasing concentrations of urea as denaturing agent. Imidazole eluted purified 70 kDa refolded protein was trypsin digested and purified by anion exchange chromatography. The activated toxin was used to check toxicity against *A. gemmatalis* first instar larvae by diet overlay method. The bioassay plates were placed a 27 °C, 70-80 % humidity and 14: 10 h light: dark photoperiod. Mortality was recorded after seven days and LC₅₀ was determined using Leora software. Cry2Ac7 found to be highly active against this pest and can be used as biopesticide.

**GENOTOXIC EFFECTS OF DIETARY BIFENTHRIN IN PERIPHERAL BLOOD
ERYTHROCYTES OF FRESH WATER FISH, *LABEO ROHITA* BY
USING COMET ASSAY**

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Bifenthrin are rapidly degraded in soil and plants, but they are extremely toxic to the fish and aquatic organisms. Fish and other aquatic organisms have the tendency to accumulate pollutants directly from contaminated water and indirectly through the ingestion of contaminated food. Keeping in view the high sensitivity of fish to bifenthrin, the present study was conducted to study the genotoxic effects of dietary bifenthrin in peripheral blood erythrocytes of freshwater fish, *Labeo rohita* through Comet assay. Fish were exposed to four sub-lethal concentrations viz. 10, 20, 33 and 50% LD₅₀ of dietary bifenthrin along with positive (Cyclophosphamide) and negative control groups, separately, under controlled laboratory conditions for 30 days. The DNA damage in terms of types of DNA damage (Type I – IV) from which percentage of DNA damaged cells, genetic damage index (GDI) and cumulative tail length of comets (μm) in peripheral erythrocytes of *Labeo rohita* exposed to different sub-lethal concentrations, with negative and positive control groups were also determined. The dietary exposure of bifenthrin to *Labeo rohita* caused significant effects on DNA damage in peripheral erythrocytes. Different concentrations of bifenthrin caused significantly variable damage to DNA with significantly higher percentage of damaged cells at 50% LD₅₀ with the mean value of 49.00±7.00 % while positive control showed the mean DNA damaged value of 42.67±1.15%. However, it was significantly lower due to negative control (2.00±2.00%). The GDI, caused by various concentrations, varied significantly also with higher GDI values at both 33% and 50% LD₅₀ exposures followed by that of positive control while negative control exhibited significantly lower mean GDI value of 0.06±0.03. The cumulative tail length of comets, induced in peripheral erythrocytes of *Labeo rohita*, was significantly larger due to positive control, followed by that of 50%, 33%, 20%, 10% of LD₅₀ and negative control. Significantly positive concentration dependent increase in DNA damage was also observed during present investigation. This study also reveals that dietary bifenthrin is very toxic to the fish and Comet assay can be used as useful tool for the determination of genotoxic effects of pesticides on fish.

A NOVEL RECOMBINANT EEL POUT (*MACROZOARCES AMERICANUS*) TYPE III ANITFREEZE PROTEIN IMPROVE CRYOSURVIVAL OF BUFFALO SPERM

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Antifreeze proteins (AFPs) are a class of polypeptides that are produced by polar fish, some species of insects, plants and microorganism. The characteristics of AFPs that attracted interest of cryobiologists include capacity to inhibit normal ice growth, ice recrystallization and membrane stabilizers. The usage of natural AFPs is associated with high costs, low yields, inadequacy of natural means, and the laborious purification processes. Recombinant manufacture of AFPs is not restricted by the seasons or natural sources, and it may be a cost-effective and less extensive source of AFPs. The objective was to study the effect of recombinant type III AFP from the eel pout *Macrozoarces americanus* (rAFPIII) in extender on post-thaw quality of buffalo bull sperm. Semen was collected from three adult Nili-Ravi buffalo (*Bubalus bubalis*) bulls of similar age group with artificial vagina (42°C) for three weeks (replicate). Semen was split into four aliquots and diluted in Tris-citric acid extender containing either 0.0 (control), 0.1, 1 and 10 µg/mL of AFGP at 37°C having approximately 50 × 10⁶ spermatozoa per mL. A fraction of this diluted semen was separated for assessment of sperm progressive motility (SPM) and sperm plasma membrane integrity (SPMI), while the remaining proportion was cooled to 4 °C in 2 hr. A fraction of this cooled semen was again separated for calculation of SPM and SPMI, while the remaining cooled semen was stabilized for 4 hr at 4 °C. Semen was filled in French straws (0.5 ml, IMV, France) by using suction pump at 4°C in the cold cabinet (Minitab, Germany). The straws were exposed to LN vapours for 10 min and stored in LN (-196°C). Thawing was performed at 37°C (30 sec.) after 24 hours. Sperm progressive motility, plasma membrane integrity, viability and acrosome intactness were assessed after thawing. There was no impact of rAFPIII on sperm hypothermic survival, as measured by SPM and SPMI, after storage at 4 °C (*P*>0.05). However, post-thaw SPM and SPMI was higher (*P*<0.05) semen supplemented with the rAFPIII (10 µg/mL) than in control samples. Sperm viability and acrosome integrity remained similar (*P* > 0.05) in extenders containing 0.1, 1 and 10 µg/mL of AFGP and control. In conclusion, the addition of rAFPIII in extender at concentration 10 µg/mL improved the freezability of buffalo sperm in terms of plasma membrane intactness and percentage of motile sperm.

ASSOCIATION OF MYOD1 SNP IN THE PAKISTANI INDIGENOUS CATTLE BREED

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MYOD1 gene belongs from the MyoD Family. It has important role in the muscle growth and development in cattle. It is involved in the determination and differentiation of muscle cell and

it is muscle specific transcription factor. In this study we identify the Single Nucleotide Polymorphism (SNP) in the MYOD1 gene to investigate the effect of polymorphism on the meat quality and to evaluate the allelic and genotypic frequencies in Pakistani indigenous cattle. We determined the novel SNP after PCR SSCP amplification the sequencing of a 299-bp fragment and mutation in the 679G>A in exon 2 of MyoD1 gene. We conclude that the SNP of the MYOD1 gene could be a very useful genetic marker for quantitative traits in cattle reproduction and breeding. This research will be helpful for the future technology.

MOLECULAR CHARACTERIZATION OF *cry11* CRYSTAL PROTEIN GENE FROM *BACILLUS THURINGIENSIS* ISOLATED FROM DIFFERENT SOIL SAMPLES

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Bacillus thuringiensis is a ubiquitous, gram-positive, spore former and produced δ -endotoxins (cry protein) which has a potential for the control of numerous lepidopteran, coleopteran, Diptera insects. The present study was aimed to control resistant insects by isolating soil samples from different localities intended for effective mosquitocidal *cry11* positive *Bacillus thuringiensis* (*B.t.*). 15 *B.t.* strains were screened, 75% were from dry, leaf liter, garden soil samples, 16% from animal waste and 10 % from moist soil of crop area. Genomic DNA was isolated and a DNA fragment of 650bp of *cry11* gene was amplified by PCR. Seven *Bacillus thuringiensis* were brought to be positive for *cry11* genes. The toxicity bioassays with *B.t.* spores proved that six *B.t.* isolates harboring *cry11* genes (*viz.*, NF1*B.t.*,2,3,4,5,6,7) were most toxic to 3rd instar larvae of mosquito, *Aedes aegypti*. Among seven *B.t.* isolates, NF5 *B.t.* 7.2, NF1*B.t.* 1.1 and NF2*B.t.* 4.2 were found the most toxic and were isolated from moist soil containing decaying cattle waste, dry waste animal dung and leaf liter soil respectively. The 16S rDNA study exposed that these screened *B.t.* confirmed 99% homology with *B.t.* serovar *tolworthi*, *B.t.* str. *Al Hakam*, *B.t.* serovar *thuringiensis*, *B.t.* serovar *konkukian*, and *B.t.* serovar *Chinensis*. *B.t.* serovar *Indiana*, *B.t.* serovar *kurstuki*. So, these isolates have a great capacity to grow into an insecticidal formulation for mosquito control.

MOLECULAR CHARACTERIZATION OF *cyt2B* PROTEIN GENE FROM *BACILLUS THURINGIENSIS*

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Bacillus thuringiensis is a gram positive, endospore producing and rod shaped entamopathogenic bacterium which is very specific in its insecticidal activity against different order of insects: Lepidoptera, Coleopteran, Diptera, Hymenoptera, Himoptera and Orthoptera. The aim of the study was to isolate *Bacillus thuringiensis* from soil and to characterize the toxic *cytB2* gene. 25 stains obtained from different localities and soil texture were screened. 55% isolates showed colony morphology and seven strains were positive for morphological and biochemical tests. Four strains were confirmed as *Bacillus thuringiensis*. GCU-DAB-M.K1 showed 99% homology with *Bacillus thuringiensis cereus*, GCU-DAB-M.K6 showed 98% homology with *Bacillus thuringiensis israelensis*, GCU-DAB-M.K10 showed 99% homology with *Bacillus thuringiensis anthracis*,

GCU-DAB-M.K15 showed 98% homology with *Bacillus thuringiensis anthracis*. Genomic DNA was isolated and a DNA fragment of 479bp of *cytB2* gene was amplified by PCR. Three *Bacillus thuringiensis* were found to be positive for *cytB2* gene. Bioassay showed that 3 stains: GCU-DAB-M.K15 (Moist soil), DAB-M.K10 (Dry Sandy soil) and GCU-DAB-M-K1 (Humid soil) have mosquitocidal activity against 3rd instar larvae of mosquito (Diptera). GCU-DAB-M.K15 showed the maximum toxicity.

THREE LOCI MOLECULAR CHARACTERIZATION OF LOCALLY ISOLATED *PARAMECIUM* SPECIES

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Like some other ciliates, *Paramecium* is being used in water quality assessment and the determination of saprobic levels. Based on the indicator values of representative species of *Paramecium*, water quality of a particular water body may be determined. Due to these and other cytological and molecular applications, an unambiguous identification of these unicellular eukaryotes is very essential. Current knowledge of the variation and diversification of these species is very limited especially in Asian countries. It is therefore need of the time to explore ecological diversity of *Paramecium* species in the local environment. For this purpose various strains of *Paramecium* belonging to different localities (Pakistan) were amplified by using five different markers i.e., 18SrDNA, ITS1-5.8S-ITS2, COXII, Hsp70 and histone H4. For our surprise one species of *Paramecium* showed its unique position in phylogenetic comparisons with the sequences already submitted in GenBank. This species was further subjected to detailed description of the life cycle, nuclear organization and sexual processes. Particularly, this is examined whether this species can support the self fertilization process of autogamy, like *P.aurelia* species or whether it is limited to conjugation, like *P. caudatum*. Another important aim was to assess whether this species show evidence of one or both of the last two whole genome duplications that have occurred in the ancestor of all *P. aurelia* species, but not in that of *P. caudatum*. This was done by counting the number of paralogs for highly, expressed, highly conserved ribosomal protein genes, all duplicates of which are usually retained after whole genome duplications. Efforts were also made to prepare the samples for the sequencing of the whole macronuclear genome of this unique species. Finally, it was also tried to find out the number of possible mating types of this species by following the light dark circadian rhythms.

ASSOCIATION OF CHILI LEAF CURL BETASATELLITE WITH *TOMATO LEAF CURL NEW DELHI VIRUS* IN PAKISTAN

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Tomato leaf curl disease (ToLCD) is a severe threat for tomato farming worldwide. In Pakistan a bipartite begomovirus *Tomato leaf curl New Delhi virus* (ToLCNDV) has been reported

as a causal agent of ToLCD. The betasatellites have been found to be associated with monopartite begomoviruses. We have found and a chili leaf curl betasatellite (ChLCB) with ToLNDV in tomato that was previously found associated with chilli. This is the first report describing the natural association of ToLCNDV with ChLCB in tomato. Thus this begomovirus betasatellite association is also a component of begomovirus complex.

**MOLECULAR CHARACTERIZATION OF COPPER RESPONSIVE
CopA, a P-Type ATPase FROM *KLEBSIELLA PNEUMONIA* KW**

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Metals are about 75% of all the known elements present on earth and can be divided into 3 sub groups: toxic, some precious metals and radionuclides. Some of these like Cu and Zn are also essential for life. Under normal conditions, both nonessential and essential metal ions may enter microbes by non specific uptake systems but microorganisms have developed specific ion efflux systems to eliminate the nonessential metals, when in excess. Metal resistance genes become up-regulated in the presence of metal ions in order to protect the cell from oxidative damage. These mechanisms include, metal exclusion by permeability barrier, active transport of the metal away from the cell/organism, intracellular sequestration of the metal by protein binding, extracellular sequestration, enzymatic detoxification of the metal to a less toxic form and reduction in metal sensitivity of cellular targets. Microorganisms use active transport mechanisms like P-type ATPases (Type-IA ATPases and Type- IB ATPases) to export toxic metals from their cytoplasm to the periplasmic space. In the present study, the cloned *copA* gene has been expressed using pET system under optimized growth conditions (17°C, 16hr incubation time and 110rpm). The expressed CopA protein was partially purified by salting out and dialysis. Its ATPase activity was measured *in-vitro*. The optimum pH and temperature for enzyme activity have been found to be 3.8 and 30°C, respectively. ATPase activity has also been found at different concentrations of substrate and enzyme. Effect of various concentrations of Cu, Cd, Zn, Ag, Pb and Au metals on the stability of expressed CopA in *E. coli* and native expression of CopA in *Klebsiella pneumoniae* KW have also been elucidated. To investigate the interaction of *pcopA* (promoter of *CopA*) with CueR, a cytoplasmic transcriptional regulator of Cue Regulon, *pcopA* has been amplified and cloned in pTZ57R cloning vector.

**ENHANCED CADMIUM BINDING ABILITY IN RESPONSE TO NOVAL
MODIFICATIONS IN A *PARAMECIUM* CADMIUM METALLOTHIONEIN PMCD1**

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Cadmium is a toxic heavy metal. Its increased concentration is detrimental to human health causing hypertension, liver damage, bone degradation and renal dysfunction. It is not only mutagenic but also highly genotoxic and carcinogenic as well. Industrial effluents are discharged to fresh water bodies which pose serious threat to living organisms. Some organisms produce

metallothioneins MTs to counter the toxic effects of non-essential metals. PMCD1 is a cysteine rich cadmium metallothionein in *Paramecium* with total of 37 cysteine residues that bind cadmium ions. Site directed mutagenesis was done to introduce new cysteine codons. The objective was to increase the number of cysteine residue and to evaluate their impact on uptake of cadmium, with far reaching effect on bioremediation. These mutations were introduced at N terminal, C terminal and in the middle of the polypeptide chain which have been respectively designated as PMCD1 N-terminal, PMCD1 C-terminal and PMCD1 int. These genes were cloned in pET21a and expressed in BL21 cells after induction with 0.1mM IPTG. Minimum inhibitory concentration (MIC) of BL21 Codon Plus cells for cadmium was determined to be 5mM. 0.5mM had the non-lethal effect on growth of cells. 1mM and 2mM lied in a sub-lethal range, whereas 3, 4 and 5mM were within near-lethal range. Maximum uptake by Atomic Absorption Spectrophotometry was observed after 24h incubation at 1mM Cd concentration rather than 2mM which was a near-lethal concentration. Unmutated PMCD1 showed the minimum uptake whereas PMCD1 N-terminal showed the drastic uptake. The uptake was observed in the order: PMCD1 N-terminal> PMCD1 mutant> PMCD1 C-terminal> PMCD1. SDS-PAGE for PMCD1 and PMCD1 N-terminal showed the stability of protein at 1mM concentration.

MOLECULAR CHARACTERIZATION OF A NOVEL METALLOTHIONEIN PMMT1 IN *PARAMECIUM* SP.

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Metallothioneins (MTs) are the cysteine rich proteins with 15-30% cysteine residues. They are produced in response to the exposure of organism with the metal ions. MTs have been identified in variety of unicellular and multicellular organisms. They have cysteine motifs in them which trap the metal ions and form metal thiolate clusters. These proteins are active in organisms living in contaminated water bodies, which help them to survive under these stressful conditions. *PMMT1* is a new metallothionein discovered in *Paramecium tetraurelia* on the basis of conserved cysteine pattern in the sequence. It was designed in such a way that TAA and TAG which are otherwise stop codons but code for glutamine in *Paramecium*, were replaced by CAA and CAG resulting in silent mutations. The deduced amino acid sequence of *PMMT1* contains 385 amino acids which include 31 cysteine residues. These residues are highly conserved in the form of repetitive structural motifs. This *PMMT1* was cloned in pET21a expression vector and expressed in *E.coli* BL21 Codon Plus strain. Maximum expression of *PMMT1* was obtained at 0.3mM IPTG concentration with 8 hours of shaking incubation. The 3-D structure of *PMMT1* using I-TASSER and Pymol indicates the conserved cysteine residues which may be in a position to make metal thiolate cluster with metal ions.

**ISOLATION AND CHARACTERIZATION OF CADMIUM RESISTANT
PARAMECIUM SP.**

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Paramecium, a free-living unicellular ciliate, can survive in a wide range of habitats like fresh water, marine water, brackish water or estuaries. *Paramecium* species tackle heavy metals by synthesizing special proteins known as metallothioneins (MTs) which bind with the heavy metal ions. Different *Paramecium* strains were collected from various areas of Punjab. The purified species was identified on the basis of histone H4 gene sequence. The isolate seemed to be identified as a new species named as *Paramecium hiraurelia*. Minimum inhibitory concentration for heavy metal cadmium was determined to be 500 μ M. Growth pattern of three strains of *Paramecium* (one *P. primaurelia* and two *P. multimicronucleatum*) was determined in the presence of different concentrations of metal ranging 10 μ M to 100 μ M. All three strains were under stress at 100 μ M concentration but they gradually adapted and continued their growth pattern till 19th day as compared to other concentrations where *Paramecium* entered the decline phase at early stage. The cadmium uptake was determined by Atomic Absorption Spectrophotometry which concludes that in the start metal uptake was observed but later metal efflux started at 100 μ M as the days passed. More uptake of cadmium was observed in *Paramecium primaurelia* (66.64ng/cell) as compared to other two species of *P. multimicronucleatum* (26.96ng/cell and 18.57ng/cell respectively). Simultaneously a metallothioneins gene *PMCD1* of 612bp was also amplified in different strains of *Paramecium*.

**COMPARATIVE STUDY TO EVALUATE THE ANTIVIRAL POTENTIAL OF
GLYCYRRHIZA GLABRA AND *FICUS BENJAMINA* LEAF EXTRACTS AGAINST
NEWCASTLE DISEASE VIRUS**

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Studies were carried out to investigate the comparative effects of crude extracts from leaves of *Glycyrrhiza glabra* and *Ficus benjamina* var. *comosa* against Newcastle disease virus (NDV) using an *in ovo* assay. Nine-day-old embryonated chicken eggs were divided into seven groups and received various treatments. Six groups were inoculated with velogenic NDV strain; five groups out of these were treated with different concentrations of the four extracts. The uninoculated and inoculated groups were left as negative and positive controls, respectively. Allantoic fluid from all treated group eggs was collected for hemagglutination test to detect NDV in the eggs. Results revealed that 600 μ g/mL aqueous extract of *Glycyrrhiza* did not produce any toxicity in the embryonated eggs and showed anti-viral activity against the virus. Similarly, 600 μ g/mL methanolic extract of *Ficus benjamina* was non-toxic in the embryonated eggs and contained anti-viral activity *in ovo*.

6. PHYSIOLOGY

THE EFFECT OF GnRH, hCG AND PROGESTERONE HORMONES ON REPRODUCTIVE PERFORMANCE OF CROSS BRED CATTLE

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The present study was designed to determine the effect of GnRH, hCG and progesterone after artificial insemination (AI) on pregnancy rate and serum progesterone concentrations in cross bred cows. A total of 40 lactating cross bred (Friesian x Sahiwal) cattle of variable lactation were selected from the herd of Military dairy farm Multan. These animals were divided in to 4 groups (A, B, C, D) each comprising of 10 animals (n=10). Group A was given a single intramuscular injection of GnRH (100 µg/animal) at day 7 after AI whereas group B was given a single intramuscular injection of hCG (3300 IU/animal) at day 7 AI cows of group C were given an intramuscular injection of progesterone (0.5 mg / kg body weight) at 7 days starting on day 7 A.I and group D was given no treatment and served as control. Blood sampling from all experimental cows was done at the start of treatments and 7 days later for progesterone estimation. All cows were examined for pregnancy by rectal palpation and its contents for detection of an embryonic vesicle on 40±1 day after AI, and pregnant cows were re-examined 4 week later on 68±1 day after AI. The mean pregnancy rate was significantly higher ($P < 0.05$) in cows of group B and C as compared to control whereas non-significant differences ($P > 0.05$) were observed between group A and D. Non-significant difference ($P > 0.05$) in conception rate was also observed between group B and C. There was non-significant difference in serum progesterone concentrations between pregnant animals of group A, B, C and D during 7 and 14 days AI, whereas 7 days after treatment, there were significant differences in serum progesterone concentrations between pregnant and non-pregnant animals of all groups (A, B, C and D). It is concluded that hCG or progesterone could be used as a method for improving pregnancy rate in Key words: Progesterone; GnRH; treatment; concentration; pregnant.

FREQUENCY OF ABO AND RH BLOOD GROUPS IN STUDENTS OF NUSRAT JAHAN COLLEGE, RABWAH, PAKISTAN

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Blood group distribution is widely used in medicine, genetic research and tracing ancestral relationships. ABO and Rh the most important blood group among all other blood group systems. Aim of the present study was to determine and document the frequency of ABO blood group and Rh factors among students of Nusrat Jahan College, Rabwah. Blood group typing was carried out during September 2016 and 385 students (325 females, 60 males) were tested. Blood group and Rh factor were determined by antigen-antibody agglutination test. The percentage of different blood

groups, 26.66% and 25.23% (blood group A), 33.33% and 36.61% (blood group B), 11.66% and 12.30% (blood group AB) and 28.33% and 25.84% (blood group O) among male and female students respectively. The Rh positive and negative distribution in the studied population was 84.67% and 15.32% respectively. ABO blood group system showed the same trend of prevalence in male and females as (B > O > A > AB). Study of blood grouping is not only generating a data base but also create social awareness about self-blood groups and help in safe blood transfusion.

MEASUREMENT OF LEVELS OF ETHINYLESTRADIOL AND TESTOSTERONE IN AGRICULTURAL AND PHARMACEUTICAL WASTE WATER IN LAHORE AND THEIR EFFECT ON *LABEO ROHITA*

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Ethinylestradiol (EE₂) and testosterone (T) hormones released in environment through effluent water of pharmaceutical and agricultural industries act as endocrine disruptor. Use of contaminated water in fish farms may seriously affect the fish health. Present study measured the levels of EE₂ and T in water samples (n= 56) collected from pharmaceutical, agricultural, and municipal waste waters. Significantly high levels ($P < 0.05$) of both hormones were measured in all samples. Moreover, effects of EE₂ and T on gonadal development were histologically studied in juvenile *Labeo rohita*. Fish were exposed to the dose of 5µg/L of EE₂ and T on daily basis for 30 days. 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.

EFFECTS OF BISPHENOL A AND BISPHENOL S AS AN ENDOCRINE DISRUPTORS ON FEMALE REPRODUCTIVE SYSTEM: A COMPARATIVE STUDY

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Endocrine disrupting potential of bisphenol A (BPA) has led to an imposition of ban on its use in infant bottles and has been replaced by alternative bisphenolic compounds. In this study effects of BPS and BPA on the female reproductive system were compared by exposing pre pubertal female rats to different concentrations for four weeks. The results showed that BPS and BPA exerted toxic effect on ovarian tissues, resulting in decreased ovarian weight and follicle number dose dependently. Catalase (CAT), super oxidase (SOD) and peroxidase (POD) level were reduced while thiobarbituric acid reactive substances (TBARS) and reactive oxygen species (ROS) were increased in treatment groups compared to control. Plasma progesterone (P4) concentration was reduced while testosterone (T) concentration increased in both treated groups. In conclusion, present study suggest that BPA and BPS exposure during pre-pubertal period, not only induces oxidative stress in ovaries but also induces alteration in follicular development.

IS ANTIMULLERIAN HORMONE (AMH) A TRUE OVARIAN MARKER FOR FIBROID, PCOS AND HYPOGONADOTROPIC HYPOGONADISM IN INFERTILE WOMEN?

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This study was designed to measure Anti mullerian hormone (AMH) concentration as a true ovarian marker in polycystic ovary syndrome (PCOS), fibroids and hypogonadotropic hypogonadism including blocked tubes, endometriosis, and unexplained primary and secondary infertile female patients. This study was prospective and performed in department of animal sciences and Salma & Kafeel medical center F-7/4 Islamabad. One hundred and twenty female patients were included. Infertile women were further divided in to seven groups and compared with fertile women. Significant increase ($p < 0.001$, $p < 0.01$) of AMH concentrations in PCOS and unexplained secondary infertile group were observed. Whereas significant decreased ($p < 0.05$, $p < 0.01$, $p < 0.05$, $p < 0.05$ respectively) were found in fibroids, HH, endometriosis and blocked tubes. High significant increase of LH, FSH concentrations ($p < 0.05$ and $p < 0.01$, respectively) was also observed in endometriosis. In unexplained secondary infertility, significant decreased ($p < 0.05$) of prolactin concentration was observed. High significant negative correlation between AMH and LH, FSH, prolactin ($R = -1$, $p < 0.001$; $R = p < 0.001$ and $R = p < 0.001$, respectively) was observed in HH and endometriosis. Whereas, high significant negative correlation between AMH and prolactin ($R = -1$, $p < 0.001$) was also observed in fibroids. It was concluded that AMH is a true ovarian marker in PCOS, fibroids, hypogonadotropic hypogonadism infertile female patients and reproductive tool to measure infertility.

BAICALEIN ALLEVIATES TESTICULAR DAMAGE IN ADULT MALE RATS CHALLENGED WITH METHOTREXATE

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Methotrexate is widely used in chemotherapy, which has been reported to induce testicular toxicity by the generation of reactive oxygen species. Baicalein is a naturally occurring bioflavonoid which is used for the treatment of many ailments. The present study was designed to evaluate the protective role of baicalein on rat testes against methotrexate. 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 methotrexate at the dose of 20mg/kg, i.p at the start of study. The third group served as a positive control and received an oral dose of (60mg baicalein/kg bw/day). In the fourth group, baicalein (60 mg/kg) was co-administered with methotrexate (20mg/kg, i.p at first day). All the treatments were carried out for 15 days. Methotrexate treatment resulted in significant ($p<0.05$) decrease in plasma and intratesticular testosterone concentrations, daily sperm production, efficiency of sperm production and number of spermatids/testis. Methotrexate treatment also induced significant ($p<0.05$) increase in the thiobarbituric acid reactive substance, whereas catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GSR) activities were significantly ($p<0.05$) decreased in testicular tissue of rat. Moreover, Methotrexate treatment significantly ($p<0.05$) induced DNA

damage. However, baicalein treatment resulted in significant ($p < 0.05$) increase in intratesticular testosterone concentrations, daily sperm production, efficiency of sperm production and number of spermatids/testis. Baicalein treatment restored antioxidant defense system and decreased the levels of thiobarbituric acid reactive substance (TBARS) significantly ($p < 0.05$). DNA damage was also recovered significantly ($p < 0.05$). Present study clearly indicates the potential protective role of baicalein against methotrexate induced reproductive damage.

STATUS OF IODINE IN DIFFERENT GROUPS OF POPULATION RESIDING IN ISLAMABAD & ADJOINING AREAS

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Iodine is a crucial nutrient for production of the thyroid gland. Iodine is obtained only through diet & mainly absorbed by the gastrointestinal track in the form of iodide. Globally 2.2 billion people (38% of the world's population) live in areas with iodine deficiency and are at risk from goiter complications. To assess the status of iodine in different groups of population residing in Islamabad & adjoining areas, three different projects were launched, summing up with results as cited below: In Project#1, Iodine Level and Thyroid Volume of 264 School Children having age in the range of 9-12 years were assessed. It was found that the girls had higher prevalence of iodine deficiency like 16.9%, 20.8% and 27.7% designated as severe, moderate and mild iodine deficiency respectively than that of boys having 16.4%, 20.2% and 29.8% iodine deficiency. Children had increased median thyroid volume with age, from 3.05 ml at 9 years to 4.36 ml at 12 years. In Project#2, Concentration of Urinary Iodide in Students and employees of PIEAS University was determined by Catalytic Reduction Method. The median urinary iodide excretion was less than 50 ug/L, indicating that population was moderately iodine deficient. In Project#3, Iodine Deficiency in 261 full term Pregnant Women was determined by catalytic reduction method and blood samples of their 125 neonates were analysed by Immunoradiometric Assay. It was found that 27% pregnant women have iodine concentration as <20ug/L (severe iodine deficiency), 50% have 20-49 ug/L (moderate iodine deficiency) and 16% have 50-99 ug/L (mild iodine deficiency). 41.8% neonates have TSH>5mIU/L, indicating low production of thyroid hormones. In all three projects, it was observed that Pakistani population residing in Islamabad and adjoining areas is iodine deficient. The best strategy to control Iodine Deficiency Disorder (IDD) in target population is salt iodization, which is one of the most cost effective ways to contribute to economic & social development.

HYPERTENSION: CONTRIBUTION OF GENETIC AND ENVIRONMENTAL FACTORS

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Contribution of genetic elements in blood pressure is around 30 to 50 % but some environmental factors (e.g. stress, dietary factors (salt, fat), temperature etc. also affect blood

pressure. It is supposed that blood pressure is due to a large number of genes, quantitative in nature and each gene has relatively slight effects. Hypertension is a multifactorial disorder, that's why it is difficult to determine the genes that produced blood pressure variation by using traditional methods that involve candidate gene studies and linkage studies. There is a developing evidence that complex interactions among multiple genes and multiple environmental factors plays a vital role in identifying an individual's risk for hypertension and for some other common diseases. Essential hypertension disturbs 20 to 30% of the population. The recent review on hypertension will be helpful in understanding the basis of hypertension, prevention, diagnosis, genetic contribution and what therapeutic agents can be used to treat hypertension. These studies can help to get the prognosis of hypertension from the symptoms and signs like headache, vision problem/impairment and fatigue etc. The prognosis helps to cure hypertension before it's severe. It can help to synthesize drugs which can be used as therapeutic agents to cure hypertension. By taking precautionary measures, like proper and healthy diet and exercise, one can prevent from hypertension. This article emphasizes to understand and solve problem related to hypertension.

IMPACT OF FLOCK RECYCLING (INDUCED MOULTING) ON PRODUCTION TRAITS OF CHICKENS

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Moulting birds replace their feathers, reduce weight, cessation of egg production takes place. Many induced moulting methods are in practice which enhance egg production, results in lower mortality and improved egg quality. Induced moulting is a world-wide practice in poultry industry which significantly contributes to profits of poultry farmers. Induced moulting enhances productivity, reduced costs and reduced industry investments in breeder farms, rearing farms, and hatcheries. Various methods of induced moulting are in practice but feed withdrawal is still considered the best method for induced moulting of hens with high profit margins throughout the world. Due to severity, high mortality rates, low immunity, criticism by animal welfare societies, public concern due to high risk of Salmonella, feed withdrawal method seems to be banned in the near future. Non feed withdrawal methods have got popularity worldwide nowadays because of better production performance in post moult period and might be practiced as an alternate induced moulting technique in future by the poultry industry. Keeping in view hen welfare concerns and future poultry industry, non feed withdrawal induced molting will be a method of choice for poultry farmers in future with added benefits.

**SUPPLEMENTATION OF MANNAN-OLIGOSACCHARIDES IN JAPANESE QUAIL:
GROWTH PERFORMANCE, VISCERA DEVELOPMENT, MINERAL
ABSORPTION AND CAECAL MICROBIOTA**

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The usage of antibiotic growth promoters (AGP) was prohibited by the European Union due to their various side effects. Prebiotics are believed to be a suitable substitute of AGP. In this connection, we conducted a study to investigate the effect of mannan-oligosaccharides (MOS) as nutritional supplementation on zoo-technical parameters, visceral organ weights, serum mineral levels and caecal concentrations of *E. coli* and *Clostridial* spp. in Japanese quail (*Coturnix coturnix japonica*). Japanese quail (one-day old; n = 1320) were taken and randomly separated into four groups (n = 320), having 8 replicates (n = 40) in each group. Corn-based poultry feed (basal diet) was given to control group birds (Group A) and the same feed mixed with three different concentrations of MOS i.e., 0.1, 0.5 and 1.0 %, was offered for 35 days to the birds of experimental groups, B, C and D, respectively. Feed intake, body weight gain (BWG) and feed conversion ratio (FCR), was monitored weekly. After completion of the experimental trial, two birds were taken from each replica and slaughtered for determination of the relative weights of different visceral organs. Serum samples were also taken to determine calcium, phosphorus, magnesium, copper, and iron levels. Caecal digseta were collected to enumerate *Escherichia coli* and *Clostridia* by culture plate method. The data were analyzed statistically using ANOVA. The results showed that the birds of group C were heavier ($P < 0.05$) during 2nd and 3rd weeks compared to the other groups. Feed conversion ratio was not affected by the dietary supplementation except on week 2, which was more ($P < 0.05$) in the group B liken with the group A. Birds of group B had higher relative weights of liver ($P = 0.57$) and gizzard ($P = 0.69$). The relative caecal weights of all MOS-supplemented groups were greater ($P = 0.10$) compared with the group A. Serum concentrations of calcium, magnesium, copper and iron were not influenced by the MOS supplementations. Serum phosphorus level was higher ($P < 0.05$) in the group B compared with the group A. The caecal *Clostridial* spp. counts were more ($P = 0.001$) in the group B compared with the other groups, while *E. coli* population remained unchanged in all groups. We found that supplementation of MOS partially influenced the growth performance of Japanese quails.

**INFLUENCE OF FEMALE BODY WEIGHT ON EXTERNAL EGG QUALITY
PARAMETERS IN TURKEYS *MELEAGRIS GALLOPAVO***

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Present experiment was conducted to find the relationship of parent body weight with external egg quality parameters in turkeys *Meleagris gallopavo*. Three pairs of turkeys, each pair comprising of one male and one female bird were kept in separate cages provided with feeding and drinking facilities for a period of three months. Each pair was offered feed of 500gm/day in feed boxes during morning hours. After 24 hours the left over feed in each box was weighed and feed intake was calculated. The eggs from each cage were collected and their weight, length and width were recorded. Body weight of the parent female showed positive correlation with egg weight, egg length and egg width of laid eggs in turkeys *Meleagris gallopavo* during present study. The weight of the eggs showed positively significant correlation with the length of the eggs.

**THE RELATIONSHIP OF PARENT BODY WEIGHT WITH EXTERNAL EGG
QUALITY PARAMETERS IN RING NECKED PHEASANTS
(*PHASIANUS COLCHICUS*) IN CAPTIVITY**

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An experiment was planned to find out the relationship of parent body weight with external egg quality parameters in ring necked pheasants in captivity. Pheasant *Phasianus colchicus* pairs of known age were arranged into three groups and were kept in cages having dimension 5×5×4 feet (length × width × height) provided with separate feeding and drinking facilities and were housed in a 20 × 20 feet (length × width) well ventilated room. Each bird was weighed and its external body measurements viz. body length, wing length, wingspan, beak length, tarsus length, thigh length, body girth and shank length were measured. All the birds were maintained under similar management conditions like temperature, humidity, ventilation, floor space and light. During present study, average egg weight of all the 30 eggs was 27.43±3.87 g, average egg length was 4.17 cm while average egg width was 3.17±0.65 cm. The correlation of parent body weight was negatively significant with the weight and breadth of the eggs. Weight of the egg showed positively significant correlation with the breadth of the eggs. Similarly, significant variations (p<0.05) in egg weight, egg length and egg breadth were recorded between all the three egg weight categories. It can be concluded from the present study, that the parent body weight and the weight of the eggs influence external egg quality parameters in *P. colchicus*.

ESTIMATION OF LIVER FUNCTION ENZYMES AND BLOOD GLUCOSE LEVEL IN BETA THALASSEMIC CHILDRENBASHIR AHMED,^{1*} ASIA PARVEEN,² RABAIL ALAM³ AND M. H. QAZI¹¹*Institute of Molecular Biology and Biotechnology, The University Of Lahore Pakistan.*²*Department of Biochemistry, University College of Medicine & Dentistry, The University of Lahore, Pakistan*³*Faculty of Life Sciences University of Central Punjab, Lahore*

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Thalassemias are a heterogeneous group of disorders with a genetically determined reduction in the rate of synthesis of one or more types of normal hemoglobin polypeptide chain. Thalassemia patients require blood transfusion on regular basis for their treatment. As a result of excessive number of blood transfusions majority of the patients develop iron overload. The present work was planned to study liver dysfunction and blood glucose estimation in patients of β -thalassemia major due to iron overload. Fifty three individuals (12 to 23 years) were selected from which 43 were beta thalassemia major patients and 10 normal individuals were used as a control. Serum ALT, AST, ALP, glucose, Hb, and Ferritin were measured. Significant increased ($p < 0.05$) was recorded in levels of ALT, AST, ALP, glucose and ferritin in β -thalassemia major patients with comparison to normal in β -thalassemia patients. Significant decrease ($p < 0.05$) was observed in hemoglobin of beta thalassemia patients as compare to control group. The outcomes of the study revealed that rise in ALT, AST, ALP, Glucose and Ferritin levels occur in thalassemics and Hb level significantly decrease in thalassemia patients.

SEROTONIN, A NOVEL DRUG FOR NEURODEGENERATION IN MOUSE MODEL

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Serotonin, a monoamine neurotransmitter, performs important functions in biological tasks such as sleep, sexual behaviour, feeding, temperature regulation, and intellect, as well as in irrational situations including obsession, anxiety, mood and pain disorders. In developing animal the blood brain barrier is incomplete and peripheral serotonin may enter the brain. Several animal studies have confirmed that enhanced activity of serotonin during development results in loss of serotonin terminals in the adults. When rats are exposed to serotonin, they show less interaction with other rats and young ones and have a reduced ability to withstand care, show modifications in hearing process They also show strong associations with dams as produce ultrasonic vocalizations when are removed from dams. They have seizures, show hypersensitivity to touch and sound and represent stereotypic motor patterns. Elevated levels of serotonin cause imbalances in oxidants and antioxidants potential in the brain, the exact mechanism of which is not known. It is the main cause of apoptosis and cell necrosis in brain. Male and female Sprague Dawley, Albino rats weighing 50 to 260 g were taken from National Institute of Health (NIH) Islamabad and kept in Animal house, Quaid-i-Azam University Islamabad. The rats were divided into two groups and were given the adjusted doses. The control group was administered normal saline and the second group received 60mg/kg serotonin for eight days. Hyperserotonemia induced loss of cellular integrity and

cytotoxicity in brain cells has been verified by histopathological analysis of haematoxylin-eosin stained slides.

CREATINE MONOHYDRATE SUPPLEMENTATION FOR 10 WEEKS HAS A POTENTIAL TO IMPROVE LEARNING AND MEMORY IN FEMALE ALBINO MICE FOLLOWING NEONATAL HYPOXIA ISCHEMIA ENCEPHALOPATHY

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Currently there are no uniform standard treatments for newborn suffering from cerebral hypoxia-ischemia (HI) and to find new and effective strategies for treating the HI injury remains a key direction for future research. Present study was designed to demonstrate that optimal dose (1 or 3%) of Creatine monohydrate (Cr) for the treatment of neonatal HI in female albino mice. On postnatal day 10, animals were subjected to left carotid artery ligation followed by 8% hypoxia for 25 minutes. Following weaning on postnatal day 20, mice were divided into three treatments on the basis of diet supplementation (Normal rodent diet, 1% and 3% creatine supplemented diet) for 10 week. A battery of neurological tests (Rota rod, open field and Morris water maze) was used to demonstrate effect of Cr supplementation on neurofunction and infarct size following HI. Open field test results indicated that Cr supplementation had significantly improved locomotory and exploratory behaviour in subjects. It was observed that Cr treated mice showed better neuromuscular coordination (rota rod) and improved spatial memory (Morris Water Maze test). A significant affect of creatine supplementation in reducing infarct size was also observed. Post hoc analysis of post hoc multiple comparisons revealed that mice supplemented with 3% Cr for 10 weeks performed better during Morris water maze test while 1% Cr supplementation improved the exploratory behaviour and gain in body weight than control group indicating that Cr supplementation has the potential to improve the neurofunction following neonatal brain damage.

VARIATIONS IN BLOOD BIOCHEMICAL PROFILE OF SOME CAPTIVE AVIAN SPECIES

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Present study was planned to record interspecific variations in blood biochemical profile of selected avian species. Significant variations ($p < 0.05$) in total protein were observed in the order peacock>pigeon>duck>rabbit>turkey> pheasant. Similarly, variations in total protein were also recorded with increase in age. Cholesterol values varied significantly ($p < 0.05$) between different avian species and maximum cholesterol values were observed from blood samples of parrot while the same were minimum in blood samples of ducks. Blood samples of peacock contained high albumin values 3.74 ± 0.42 mg/dl while pheasant and parrots blood samples had lower albumin

values. Significantly ($p < 0.05$) higher globulin concentrations were observed from blood samples of peacock while non-significant variations in globulin were recorded between ducks, turkeys and pheasant's blood samples. During present study, total protein values varied between 4 mg/dl to 10 mg/dl in all birds except blood samples of peacock. Similarly, gender-wise variations were recorded in glucose concentrations between male and female birds.

**LONG TERM CREATINE MONOHYDRATE SUPPLEMENTATION, FOLLOWING
NEONATAL HYPOXIC ISCHEMIC INSULT, IMPROVES NEUROMUSCULAR
COORDINATION AND SPATIAL LEARNING IN MALE ALBINO MOUSE**

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Creatine is known to rescue animals following brain damage. Present study was designed to demonstrate the effect of long term (15 week) supplementation of 2% creatine monohydrate (Cr), following neonatal hypoxic ischemic insult, on learning and memory formation in male albino mouse. Albino mice pups were subjected to right common carotid artery ligation followed by 8% hypoxia for 25 minutes. Following weaning, animals were separated and grouped on the basis of dietary supplementation for 15 weeks followed by a battery of neurological tests including Morris water maze, open field and rota rod. It was observed that HI mice fed on 2% Cr for 15 weeks performed better than their littermates mice on normal rodent diet during water maze (learning and memory) and rotating rod (neuro-muscular coordination and balance) test while the results of open field test remained unaffected. It was also observed that Cr treated animals had a reduced brain infarct volume than untreated but this difference did not reached statistical significance. We have also observed an overall increase in body weight in Cr treated mice during the study. Over all our results are indicating that long term Cr supplementation is beneficial for male albino following hypoxic ischemic insult.

**COMPARATIVE EFFICACY OF NATURAL AND SYNTHETIC ANTI STRESSORS
ON CARCASS CHARACTERISTICS IN BROILERS SUBJECTED TO
CYCLIC HEAT STRESS**

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Heat stress is one of the major threat for Poultry industry. A study was conducted with the objective to compare efficacy of different anti stressor for carcass yield in broilers subjected to cyclic heat stress. A total of 360 birds were subjected to 5 treatments (Control, Vitamin C @ 650mg/Kg, Betain @ 3g/Kg, Turmeric Powder @ 5g/Kg, Grounded Chia seed @ 5g/Kg) divided into 6 replicates of 12 bird each. During last two weeks the birds were subjected to cyclic heat

stress of temperature and humidity i.e., $34\pm 1^{\circ}\text{C}$ and $75\pm 5\%$, respectively for 8 hours. After 5 weeks, 10 birds from each treatment were randomly picked, transported to 80 kilometer and slaughtered manually using Halal method. The eviscerated carcass was cut into different parts for carcass cut up yield. The data collected were subjected to Analysis of Variance technique and significant means were compared using Duncan's Multiple Range test. The results showed that result showed significant difference among different treatments for weight loss during live hauling, Head %, Abdominal fat %, Water uptake during chilling, neck %, and breast %. While, shank%, carcass %, giblets, blood loss %, wing %, drumstick %, thigh %, fillet %, ribs and back % remained non-significant.

GLUCOSE AND LIPID PROFILE IN PREGNANCY INDUCED HYPERTENSION IN A LOCAL POPULATION: A CASE CONTROL STUDY

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To estimate the lipid profile and glucose level in pregnancy induced hypertensive verses normal pregnant women in local population of Pakistan. Cross Sectional case control study. All biochemical samples were analyzed in Institute of Molecular Biology and Biotechnology (IMBB), UOL from March to December 2012 at Gynecology department of Jinnah hospital Lahore. Total of 60 individuals who consented was divided into case and control groups. The cases included 30 hypertensive pregnant women and controls included 30 normal pregnant women blood samples were collected from Gynecology department Jinnah hospital Lahore. Detailed physical examination were performed blood pressure, age and weight were record. The glucose level was found to be slightly high in hypertensive pregnant women but no significant change was observed in hypertensive (Mean \pm SD: 95.93 ± 14.268 vs. 87.86 ± 17.83). The total cholesterol was found to be significantly higher in hypertensive pregnant women as compared to normal pregnant women (274.16 ± 37.55 vs. 171.00 ± 30.776). The TG level significant high in hypertensive then normal group (175.06 ± 32.20 vs. 114.86 ± 20.58) and HDL level low in hypertensive group (49.4 ± 8.15 vs. 55.42 ± 7.98). The LDL level (171.66 ± 29.98 vs 105.60 ± 19.66) and VLDL level (34.90 ± 6.47 vs. 22.83 ± 4.06). The women with very high BP had higher Cholesterol, TG, LDL and VDL is also high in hypertensive pregnant women. In normal pregnant women the level of cholesterol, TG, HDL, LDL and VLDL are normal or slightly raised. In, glucose level is considered a non - significant risk factor for hypertension.

EVALUATION OF SERUM LIPID PROFILE AND METAL ION ABNORMALITIES IN BETA THALASSEMIC CHILDREN OF PAKISTAN

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The present project was designed to evaluate the lipid profile and metal ion abnormalities in β - thalassemic children in Pakistani population. From July to December 2014, 65 consecutive

children with β -thalassemia were enrolled for study by visiting the thalassaemia Center at Sundas Foundation 880 shadman Lahore, every month for routine examinations. Fasting blood lipid profile, serum Ferritin and Hb were measured in all participants. Calcium and Magnesium levels in serums were determined. The commercially available kits by humans & S bio were used for all analysis. Out of 65 patients, 43 were males and 22 were females. Data analysis showed that mean levels of HDL, LDL, VLDL, TG and cholesterol were recorded in beta thalassaemic children as 44.85 ± 8.05 mg/dl, 97.55 ± 55.36 mg/dl, 38.04 ± 11.04 mg/dl, 190.25 ± 54.61 mg/dl and 1177.55 ± 56.11 respectively. Ferritin and Hb levels were also observed as 3295.03 ug/L ± 1780.29 g/dl and 6.78 ± 1.35 respectively. About 80% patients showed low serum level of Ca⁺. These all test were also performed for 10 control individuals. The majority of the patients had blood lipid levels within the normal range, and consequently the prevalence of lipid abnormalities was low as compared to the normal population of the same age. Interestingly, the total Ferritin and cholesterol levels were very high in our patients, and may underline the importance of this index for the prognosis of future cardiac events and adverse effect on other organs in these patients. The serum Ca⁺ levels were low in most of the patients which may cause some metabolic abnormalities in future.

ASSESSMENT OF HEPATOPROTECTIVE AND NEPHROPROTECTIVE POTENTIAL OF POLYDATIN ON BROMOBENZENE INDUCED INJURY IN RATS

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Bromobenzene is a renowned environmental toxin which causes hepatic and renal damage through CYP450 mediated bioactivation to cause oxidative insult. In this study, protective effects of polydatin were evaluated against bromobenzene induced injury. Forty adult male Sprague-Dawley rats were randomly distributed into four equal groups (n=10/group). The first group was provided with normal saline. The second group of rats was treated with bromobenzene at the dose of 15 mmol/kg at the start of study. The third group served as a positive control and received an oral dose of (100 mg polydatin/kg bw/day). Fourth group was co-treated with (100 mg/kg polydatin + 15 mmol/kg bromobenzene). All the treatments were carried out for 45 days. Bromobenzene treatment resulted in significant (p<0.05) increase in the thiobarbituric acid reactive substance, whereas catalase (CAT), peroxidase (POD) and glutathione reductase (GSR) activities were significantly (p<0.05) decreased in hepatic and renal tissue of rat, superoxide dismutase (SOD) activity remained unchanged. Moreover, bromobenzene treatment also significantly (p<0.05) induced DNA fragmentation. However, polydatin treatment restored antioxidant defense system and decreased the levels of thiobarbituric acid reactive substance (TBARS) significantly (p<0.05). DNA damage was also significantly (p<0.05) recovered followed by polydatin treatment. Therefore, it was concluded that polydatin can be used as a therapeutic drug against bromobenzene induced damage in liver and kidney.

**ROLE OF PROBIOTICS SUPPLEMENTATION ON GROWTH PERFORMANCE OF
LABEO ROHITA FINGERLINGS FED SUNFLOWER MEAL BASED DIETS**

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Present research work was conducted to evaluate the effect of probiotics supplementation on growth performance of *Labeo rohita* fingerlings fed Sunflower meal (SFM) based diets. Graded levels (0 gKg⁻¹, 0.5 gKg⁻¹, 1 gKg⁻¹, 1.5 gKg⁻¹, 2 gKg⁻¹, 2.5 gKg⁻¹ and 3 gKg⁻¹) of probiotics were supplemented in fish feed to formulate one control and six test diets. SFM was used as test ingredient to formulate experimental diet. Fifteen fingerlings were stocked in each replicates. The fingerlings were fed at the rate of 5% of live wet fish body weight on their prescribed diet twice daily. Present results showed that supplementation of probiotics in SFM based diets significantly ($p < 0.05$) enhanced growth performance of fingerlings as compared to control diet. *L. rohita* fingerlings fed SFM based diet supplemented with 2 gKg⁻¹ level of probiotics indicate a significant improvement ($p < 0.05$) in weight gain (22g), weight gain% (307%), SGR (1.56) and minimum FCR (1.21) as compared to fish fed on control diet (weight gain 17g, weight gain% 242%, SGR 1.37 and lowest FCR 1.69). It was concluded that 2 gKg⁻¹ level of probiotics supplementation in SFM based diet is the suitable for improving growth performance of *L. rohita* fingerlings.

**CIRCULATING INFLAMMATORY ADIPOKINES: POTENTIAL BIOMARKERS IN
NON-DIABETIC OBESE INDIVIDUAL**

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Obesity is anomalous or excessive fat accretion that presents a major health threat according to the WHO. This aberrant adipokine production from abnormal adipose tissue accumulation leading to abnormal metabolic inflammation and changes in body homeostasis are the major cause of obesity related health problems. The aim of this study was to evaluate role of inflammatory and anti-inflammatory adipokines as biomarkers in obesity and their potential role in obesity associated co-morbidities. The objectives of this study are to estimate the serum level of IL-6, TNF- α , Resistin and adiponectin in non-diabetic obese subjects as markers of inflammation, using Luminex-based multiplex cytokine assay. Furthermore, to correlate serum adipokines levels with tobacco habits in obese patients. In this study 33 non-diabetic obese patients of both gender, aged 18-45 years, having body mass index (BMI) equal to or greater than 30 kg/m² and 30 lean, age and gender-matched individuals with BMI 18.5-24.5 kg/m² were targeted for participation. The serum adipokine levels were evaluated in each sample using Luminex multianalyte profiling (xMAP) technology on BioPlex instrument. Our results showed that serum concentrations of TNF- α was higher in control individuals than obese patients. While, that of adiponectin in obese patients

was lower than age and gender matched healthy control subjects with statistically significant difference ($p=0.04$ & $p=0.000$, respectively). Moreover, we have found some gender-based differences in IL-6, TNF- α and Resistin levels. Control male individuals were found to have higher values for serum IL-6, TNF- α and Resistin than obese males, where the difference was statistically significant. Furthermore, IL-6, TNF- α and Resistin showed higher levels in control tobacco smokers than obese smokers with statistically significant difference in case of TNF- α ($p=0.009$). TNF- α and Resistin levels were also found to differ with statistically significant difference between control tobacco users and control non-tobacco users ($p=0.002$ & $p=0.014$, respectively). Adiponectin did not show any tobacco dependent profiles in both control and obese subjects. IL-6 correlated positively with TNF- α and Resistin in control group ($p<0.01$ & $p=0.03$). In same group, TNF- α positively correlate with Resistin ($p=0.007$) and TNF- α negatively correlates with Adiponectin ($p=0.030$). Our results conclude that adipokine levels can be successfully monitored in body fluids, in our case, serum. We have found interesting differences in the serum concentration of various adipokines studied, which reflects the inflammatory status of individuals suffering from obesity. Our results concluded that tobacco use suppresses the serum levels of IL-6, TNF- α and Resistin in obese males and therefore hides probable effects of smoking on systemic inflammatory adipokine levels in these individuals. Further studies are needed to explore the levels of serum adipokines in diabetic obese subjects with and without tobacco use, in order to study disease progression and prevent obesity associated co-morbidities, especially related to inflammatory adipokines.

EFFICACY OF PROBIOTICS SUPPLEMENTATION ON CARCASS COMPOSITION AND HEMATOLOGICAL PARAMETERS OF *CYPRINUS CARPIO* FINGERLINGS FED CORN GLUTEN MEAL BASED DIETS

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This research work was conducted to evaluate the effect of probiotics supplementation on carcass composition and hematological study of *Cyprinus carpio* fingerlings fed corn gluten meal based diets. Graded levels (0 gKg⁻¹, 1 gKg⁻¹, 2 gKg⁻¹, 3 gKg⁻¹, 4 gKg⁻¹ and 5 gKg⁻¹) of probiotics were supplemented in fish feed to formulate one control and five test diets. Three replicates were used in the present study for each treatment and in each replicate 15 fingerlings were stocked. The fingerlings were fed at the rate of 5% of live wet weight on their prescribed diet twice daily. The results showed that supplementation of probiotics in corn gluten meal based diets significantly ($p<0.05$) enhanced carcass composition and hematological parameters of fingerlings as compared to control diet. *C. carpio* fingerlings fed corn gluten meal based diet supplemented with 2 gKg⁻¹ level of probiotics indicated significant improvements ($p<0.05$) in crude protein (17g) crude fat (9g) and gross energy (3 kcal g⁻¹) whereas higher RBCs WBCs and Hb was also recorded in fish fed 2 gKg⁻¹ level of probiotics. From these results, it was concluded that 2 gKg⁻¹ level of probiotics

supplementation in corn gluten meal based diet is the optimum for improving body composition and hematology of *C. carpio* fingerlings.

EFFECT OF AGE ON HEMATOLOGICAL PARAMETERS IN RING NECKED PHEASANT IN CAPTIVITY

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The aim of the study was to investigate the effect of age on haematological profile in ring-necked pheasant (*Phasianus colchicus*) in captivity. The study was conducted at Wildlife and Ecology department of University of Veterinary and Animal Sciences C-block Ravi campus, Pattoki. A total of 130 birds of different age groups from chick to adult were allocated in experimental floor-pen housed, receiving water and poultry feed ad libitum. The different parameters of blood i.e Red blood cell, White blood cell, Haemoglobin amount, mean corpuscular haemoglobin concentration, total serum protein, Heterophils, lymphocytes, monocytes, Eosinophils and basophils were counted at 6th, 8th, 9th, 11th, 12th, 14th, 15th, 17th, 18th, 20st, 21th, 24th weeks and one year of age. Statistical analysis showed the significant difference with the reference of age groups. Maximum value for RBC was observed in 11th and 24th week of age while significant increase in WBC was observed in 8th week of age. Highly significant value for hemoglobin amount was observed in 15th week of age. Maximum values for Packed cell volume and total serum protein were observed in 20th, 21st, 24th and one year of age. Heterophils and lymphocytes showed the maximum increase in 8th week of age while monocytes showed the maximum increase in 20th, 21st, 24th and one year of age. Eosinophils increase in 21st week of age while basophils showed maximum increase in 6th week of age.

CRYOPROTECTIVE EFFECT OF GLYCEROL CONCENTRATIONS ON INDIAN RED JUNGLE FOWL (*GALLUS GALLUS MURGHI*) SPERMATOZOA

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Semen cryopreservation protocols for wild avian species require to be optimized to achieve optimum post-thaw sperm quality and fertility. The present study was designed to evaluate the cryoprotective effect of different glycerol concentrations (11%, 15% and 20%) on post-thaw quality, recovery rates, absolute livability index and fertility attributes (number of fertile eggs, percent fertility, no. of hatched chicks, percent hatch and hatchability of fertilized eggs) of Indian red jungle fowl semen. Semen was collected from eight mature cocks and cryopreserved for storage at -196°C. Frozen semen was thawed at 37 °C for 30 seconds and assessed for motility, plasma membrane integrity, viability and acrosome integrity at 0, 2 and 4 hours incubation at 37°C.

Percentages of motility, plasma membrane integrity, viability and acrosome integrity were recorded higher ($P < 0.05$) post-thaw at 0, 2 and 4 hours at 37°C with glycerol 20% compared to 15% and 11% glycerol. Likewise, recovery rates (%) of aforementioned parameters after cryopreservation and absolute livability index were observed highest ($P < 0.05$) with 20% glycerol. By comparing values of R^2 after multivariate regression analysis, least negative effects of hours of incubation were observed on semen quality in extender with 20% glycerol followed by 15% and 11% glycerol. The fertility outcomes (number of fertile eggs, fertility (%), no. of hatched chicks, percent hatch and hatchability of fertilized eggs) were recorded higher ($P < 0.05$) with 20% glycerol followed by 15% and 11% glycerol. It is concluded that the concentration of 20% glycerol shows the best cryoprotective ability for quality and fertility of Indian red jungle fowl semen.

EFFICACY OF PROBIOTICS SUPPLEMENTATION ON MINERAL DIGESTIBILITY AND CARCASS COMPOSITION OF *Oreochromis niloticus* FINGERLINGS FED CANOLA MEAL BASED DIETS

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Present trial was conducted to evaluate effects of probiotics on mineral digestibility and carcass composition of *Oreochromis niloticus* fingerlings fed canola meal-based diet. Six test diets (0, 1, 2, 3, 4 and 5 g Kg⁻¹ level) were prepared using probiotics (Protexin) in the canola meal based diet. Triplicate tanks were used for each treatment and 15 fingerlings were stocked in each tank. Fingerlings were fed at the rate of 5 % of live wet weight. Plant meal based diets played negative effect on fish growth performance in the absence of probiotics. Chromic oxide (1%) was added in the diets as inert marker. It was noted that probiotics supplementation played a significant role in improving ADC% of minerals and carcass composition of fish. Higher mineral digestibility (Ca 76%, Fe 62%, Zn 57%, P 76%) and carcass composition (Crude protein 17% and crude fat 10%) calculated in the fish fed 3 g Kg⁻¹ level of probiotics supplemented in canola meal based diet. Whereas higher ADC% of Na (75%), K (67%) and Mg (62%) was recorded in the fish fed at 4 g Kg⁻¹ level of probiotics supplemented in canola meal based diet. On the basis of these results it was concluded that probiotics supplementation at 3 g Kg⁻¹ level in canola meal based diet is helpful for maximum performance of *Oreochromis niloticus* fingerlings.

DETERMINATION OF DISPOSITION KINETICS AND URINARY EXCRETION OF CEFADROXIL IN FEMALE PATIENTS SUFFERING FROM RENAL IMPAIRMENT

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Cefadroxil is a first generation aminocephalosporin antibiotic, commonly used to treat serious diseases caused by susceptible strains of microorganisms in lower respiratory tract

infections, urinogenital infections, gynaecologic infections and skin infections. Disposition kinetics and urinary excretion of cefadroxil were investigated in 20-30 years old nine female patients suffering from renal impairment having serum creatinine level 3-10 mg/dL and blood urea nitrogen 100-300 mg/dL. The cefadroxil capsule having brand name Ibdroxil (500mg, Glaxo Smith Kline Pvt, Pakistan) was given orally as a single dose. Blood and urine samples were analysed on high performance liquid chromatography (HPLC) system for Cefadroxil drug concentration. The APO system was used to study various pharmacokinetics parameters. Urinary excretion was expressed as cumulative percent of dose excreted in the urine. The statistical regression analysis was performed. Cefadroxil plasma concentration at 1 hour was 19.71 ± 14.41 $\mu\text{g/ml}$. After 3 hours, it reached at maximum concentration of 27.57 ± 0.61 $\mu\text{g/ml}$ and then declined with the passage of time, at last it reached at 12.44 ± 1.64 $\mu\text{g/ml}$ at 12 hour. The maximum concentration of single dose of Cefadroxil 500mg after oral administration in the female renal impaired patients was 25.08 ± 1.00 $\mu\text{g/ml}$. Cmax was achieved at 3 hours under the mean range of 24.08-26.09 $\mu\text{g/ml}$. The time for the peak concentration for cefadroxil ranged from 2.58-2.80 hours with mean \pm S.E. 2.69 ± 0.10 hours. The elimination half life ranged from 7.38-12.48 hours with 9.93 ± 2.55 hours. The apparent volume of distribution of drug was 0.27 ± 0.01 per kg. The mean residence time for single dose of cefadroxil 500mg after oral administration ranged from 11.96-19.08 hour of with mean \pm S.E. 15.52 ± 3.55 hours. Urinary excretion of cefadroxil calculated as mean cumulative percentage of dose excreted in urine was 0.94 ± 0.07 percent at 12 hours. The study highlighted that there are significant differences in several pharmacokinetic parameters (Cmax, Tmax, Vd etc.) between female patients with renal impairment as compared to healthy individuals. Suggested dose regimen is 447.38 mg as loading dose and 164.58mg as maintenance dose with 12 hours time interval in renal impaired female patients.

EVALUATION OF HONEY AS ALTERNATIVE TO ANTIBIOTICS IN EXTENDER FOR CRYOPRESERVATION OF BUFFALO BULL SEMEN

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The aim of this study was to investigate the efficiency of honey as alternative to antibiotics in extender for cryopreserved Nili Ravi buffalo semen. For this purpose, honey collected from different plant sources viz., *Zizhipus jujube*, *Acacia nilotica* and *Brassica campestris* were evaluated. The composition of *Zizhipus* honey was found suitable in terms of moisture content, total sugars, sucrose content, pH, electrical conductivity, acidity and density. In vitro tolerability of buffalo sperm for honey was assessed using sodium citrate buffer containing different levels of honey (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1%). Honey up to 0.2% was found nontoxic to motility and viability of buffalo sperm. To evaluate honey for cryopreservation of buffalo sperm, tris-citric egg yolk extender was used as control; while experimental extenders included honey at 0.1%, 0.2%; honey (0.1% or 0.2 %) without fructose; honey (0.1% or 0.2 %) without antibiotics; honey (0.1% or 0.2 %) without fructose and antibiotics. Semen collected from buffalo bulls (n=3) was cryopreserved and the experiment was repeated three times (replicates). Post-thaw sperm motility (%), plasma membrane integrity (%), viability (%) and livability (%) was higher (P<0.05)

in extender containing 0.2% honey without antibiotics. Total aerobic bacterial count was 0.00 in extender containing 0.2% honey and fructose without antibiotics, compared with 4.7×10^3 CFU/ml in extender without fructose and antibiotics. In conclusion, 0.2% honey could be considered as an alternative to antibiotics in tris-citric extender for cryopreserved buffalo sperm.

EFFECT OF VITAMIN E IN EXTENDER ON CRYOPRESERVATION OF INDIAN RED JUNGLE FOWL SPERM

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Cryopreservation of avian sperm is associated with oxidative stress that resulted in reduced functional and structural integrity of sperm after freeze-thawing. The objective of the study was to determine the effect of Vitamin E supplementation in red fowl extender on quality of Indian red jungle fowl semen at different stages of cryopreservation. Semen was collected from eight mature cocks of red jungle fowl and assessed initially for semen volume, motility and concentration. The ejaculates having >60% motility were pooled, divided into aliquots and diluted with extender having Vitamin E 0.0% (control), 0.5%, 1%, 2% and 3%. Semen was cryopreserved using standard procedures. Vitamin E supplementation of extender did not affect motility, plasma membrane integrity, viability and acrosomal integrity of cryopreserved semen. However, the stages of cryopreservation had negative effect on aforementioned parameters irrespective of extenders. It is concluded that Vitamin E supplementation of red fowl extender did not improve the quality of cryopreserved Indian red jungle fowl semen.

EFFECT OF CITRIC ACID, PHYTASE AND ORGANIC TRACE ELEMENTS SUPPLEMENTATION ON GROWTH PERFORMANCE, NUTRIENTS DIGESTIBILITY AND DIGESTIVE ENZYME ACTIVITIES OF *LABEO ROHITA* FINGERLINGS

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The present study with *Labeo rohita* fingerlings was designed to investigate the effect of citric acid (CA), phytase (PHY) and organically chelated trace elements (TEs) supplementations in sunflower meal (SFM) based diets. The three supplements i.e. CA (0 and 3%), PHY (0 and 1000 FTU/kg) and TEs {(inorganic (0.15%) and organic (0.1% and 0.05%))}, were supplemented in factorial arrangement ($2 \times 2 \times 3$) resulting in the formulation of 12 treatments. Fish were fed in triplicates to apparent satiation twice a day, 6 days a week. Fecal matter was collected daily for determination of digestibility. Fish were weighed fortnightly for the record of growth performance. At the end of feeding trial, fish was harvested and intestine of fish from each treatment was

collected for determination of digestive enzyme activities. Independent effects of all the three supplements resulted in improved growth performance of fingerlings. Likewise, feed intake and feed conversion ratio (FCR) were also affected positively by the supplementation of these feed additives. Dietary acidification through CA, PHY and organic TEs supplementation improved ($p < 0.05$) the digestibility of dry matter, crude protein, crude fats and gross energy. Similarly, CA, PHY and organically chelated TEs addition also enhanced ($p < 0.05$) the mineral absorption. Activities of intestinal amylase, protease and lipase were significantly ($p < 0.05$) enhanced by dietary additives (CA, PHY and TEs) in *L. rohita* fingerlings. The second order interactions (CA×PHY, CA×TEs, TEs×PHY) were mostly significant for all observed parameters, However, the higher order interactions (CA×PHY×TEs) were only found significant ($p < 0.05$) for FCR and major mineral absorption. Conclusively, supplementation of CA, PHY and organically chelated TEs improved the growth performance, nutrient digestibility, mineral absorption and intestinal digestive enzyme activities with significantly positive second order interactions.

LINOLEIC ACID SUPPLEMENTATION IN EXTENDER IMPROVES SPERM PROGRESSIVE MOTILITY AND PLASMA MEMBRANE INTEGRITY OF CRYOPRESERVED NILI-RAVI BUFFALO SPERMATOZOA

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In buffalo sperm total lipids and phospholipids are lost during cryopreservation and this loss can be mitigated by enriching the extender with fatty acids that can improve the plasma membrane stability of cryopreserved buffalo sperm. The aim of present study was to evaluate the effect of linoleic acid supplementation in extender on post thaw quality of cryopreserved Nili-Ravi buffalo bull semen. For this purpose semen was collected from three adult Nili-Ravi buffalo bulls of similar age with artificial vagina (42°C) for five weeks (replicate; N=30). Semen was split into four aliquots and diluted in *tris*-citric acid extender containing 0.0 (control), 5.0, 10.0 and 20.0 ng mL⁻¹ of linoleic acid at 37°C having approximately 50×10^6 spermatozoa mL⁻¹. Diluted semen was cooled to 4°C in 2 hours and equilibrated for 4 hours at 4°C. Cooled semen was filled in 0.5 ml straws at 4°C, kept on liquid nitrogen vapours for 10 min. and plunged in liquid nitrogen for storage. Thawing of frozen semen was performed after 24 hours at 37°C for 30 seconds and assessed for semen quality parameters. Sperm progressive motility and structural and functional integrity of sperm plasma membrane was improved ($P < 0.05$) in extender containing 10.0 ng mL⁻¹ of linoleic acid compared to other treatments and control while linoleic acid supplementation didn't provide any significant benefit for number of acrosome intact live sperm and chromatin intact spermatozoa. In conclusion, linoleic acid supplementation in extender at 10.0 ng mL⁻¹ of extender improved the motility and structural and functional integrity of sperm plasma membrane in buffalo.

**IN VITRO LONGEVITY OF CRYOPRESERVED SEMEN FROM
VARIOUS CATTLE BREEDS**

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The aim of the present study was to compare *in vitro* longevity of cryopreserved semen from different cattle breeds viz., Cholistani, Dhanni, Jersey, Holstien Friesien and Sahiwal. Semen was collected from three mature bulls of each breed belonging to similar age group for a period of three weeks (replicates). The qualified semen ejaculates (>1 mL volume, >60% motility, >0.5 billion/mL concentration) were cryopreserved in *Tris* citric extender using standard procedure. After 24 hours of freezing, semen was thawed in a water bath (37°C) for 30 sec and incubated at 37°C for further evaluation. The *in vitro* longevity was assessed in terms of sperm progressive motility, plasma membrane integrity and viability at hourly intervals. In Cholistani breed, there was only 8% loss of motility with every hour of incubation. While 10-12% loss of motility was observed in other breeds. In terms of sperm plasma membrane integrity, Cholistani, Dhanni and Friesien breeds showed only 7% decrease with every hour of incubation. However 8% and 9% decrease of sperm plasma membrane integrity was observed in Jersey and Sahiwal breed. Cholistani breed was found better in terms of sperm viability as only 3% loss of viability/hour of incubation was observed. Maximum loss of viability (8%) was observed in Sahiwal breed. In conclusion, *in vitro* longevity of cryopreserved semen in terms of semen quality parameters was higher (P<0.05) in Cholistani breed compared to Jersey, Friesien, Sahiwal, and Dhanni.

**EFFECT OF ANTIBIOTICS IN EXTENDER ON BACTERIAL CONTROL AND
SPERMATOZOAL QUALITY OF INDIAN RED JUNGLE FOWL
(GALLUS GALLUS MURGHI)**

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The study was designed to evaluate the effect of various antibiotics on bacterial control and semen quality of Indian red jungle fowl (*Gallus gallus murghi*). Semen ejaculates were collected from eight cocks of Indian red jungle fowl (N=8), pooled and divided into six portions and cryopreserved with Beltsville poultry semen extender containing antibiotics viz; streptomycin, neomycin, kanamycin, gentamycin, penicillin and control (without antibiotic). Sperm motility, plasma membrane integrity, livability and acrosomal integrity were observed at post dilution, post cooling, post equilibration and post thawing. The study was replicated for five times. None of the antibiotics addition in extender showed significant negative impact (P>0.05) on semen quality parameters compared to control. The bacterial contaminants isolated were *Escherichia coli*, *Staphylococcus spp.* and *Bacillus spp.* All of these bacteria were found sensitive to penicillin.

Staphylococcus spp. was sensitive to streptomycin, penicillin and neomycin. *Bacillus* spp. was found sensitive to penicillin and gentamycin. It is concluded that penicillin can be used in extender for bacterial control efficiently without comprising semen quality of Indian red jungle fowl.

PRIMILINARY STUDY OF AFFINITY OF ESTROGEN ON MOOD SWINGS AMONG FEMALES

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The name estrogen comes from the Greek *οἶστρος* (oistros), literally meaning "verve or inspiration" but figuratively sexual passion or desire, and the suffix *-gen*, meaning "producer of". Like all steroid hormones, estrogens readily diffuse across the cell membrane. Once inside the cell, they bind to and activate estrogen receptors (ERs) which in turn modulate the expression of many genes. During the present study **mood swings** of 25 college girls were observed with range of age in between 13-20 years, through questionnaire and blood investigation of estrogen level in two phases. Data was collected from January to June 2016. Phase I showed observation before menstruation and Phase II showed observation during menstruation. In results of phase I; 64% girls showed irritability, 57% showed headache, 56% showed tension, 48% showed sleep problem and lack of motivation, 44 % showed mental confusion 32% showed depressive mode and anger. While in results of phase II; 84% showed increase fatigue, 80% showed tension, sleep problem and mood change behavior, 76% showed irrational anger, 68% mental confusion, 64% headache, 52% depressive mood and 44% lack of motivation. Results clearly indicated that **ESTROGEN** levels have strong impact on mood swings in girls.

A COMPARATIVE STUDY OF PLASMA IRISIN CONCENTRATIONS AND REPRODUCTIVE PARAMETERS AMONG EXPLAINED INFERTILE, UNEXPLAINED INFERTILE AND FERTILE WOMEN

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Irisin is a novel myokine that is produced as a result of exercise. The proteolytic cleavage of Fibronectin type III domain containing 5 protein (FNDC5) by an unknown protease results in the release of irisin into blood circulation. Irisin causes the conversion of white adipocytes into brown adipocytes and consequently results in increased heat production and energy expenditure. Previous studies have confirmed irisin presence in central as well as in peripheral tissues. Many studies reveal metabolic roles of irisin but a very few studies show that irisin is a possible metabolic marker in reproduction. The aim of the present study is to measure the levels of irisin in explained infertile, unexplained infertile and fertile women. Secondly, study was done to find out association of irisin with anthropometric parameters, lipid profiles, reproductive and metabolic hormones. The blood samples of 104 women included in the study, were collected to measure irisin, LH, FSH, PRL, AMH, TSH, T₃, T₄, glucose, HDL-c, LDL-c, total cholesterol and triglycerides. Similarly, anthropometric characteristics of the women were noted as well. All the

data is expressed as mean \pm SEM. Unpaired t-test and one way analysis of variance (ANOVA) followed by Tukey's test were used to compare the data among groups. The association of irisin with anthropometric, reproductive, lipid and metabolic parameters was assessed by Pearson's correlation. Both unexplained infertile and explained infertile women showed highly significant ($p < 0.001$) low levels of plasma irisin than those of control. Irisin showed a significant inverse correlation with Age, BMI and Prolactin while, a significant positive correlation with LH, Blood Glucose, Triglycerides, LDL-c and HDL-c. The existing study confirms the significant low levels of irisin in unexplained infertile and explained infertile women compared with fertile women. As hypothyroidism, endometriosis, fallopian tubes blockage and uterine fibroids are considered as risk factors of infertility, we can speculate that lower levels of irisin also might be the possible indicator of infertility. So, by increasing the levels of irisin in such patients may improve their infertility problems.

**MODULATION OF MICRO- AND MACRO-ELEMENT CONCENTRATIONS
FOLLOWING ACUTE AND CHRONIC ADMINISTRATION OF *NIGELLA SATIVA* L.
OIL ON RAT BODY TISSUES**

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Nigella sativa (N. sativa), a small herb of Ranunculaceae family is considered a miracle herb. It is used as an antibacterial, antiviral, antifungal, anaparasitic, anti-inflammatory, anticancerous, cardioprotective, nephroprotective, antidiabetic, antihistaminic and immunodulatory agent. Micro- and macroelements play diverse roles in the physiology of an organism to run cellular and subcellular functions and to communicate with vitamins. They also regulate production of hormones, facilitate the metabolic processes, electricity generation, nerve conduction and regulation of gene expression. Elemental concentrations of the body are although very well-known and the effects as a result of their deficiency or excess are also very well established but the role of common herbs like *Nigella sativa* in the regulation of elemental concentrations has not been reported previously. The current study was carried out to investigate the effect of *Nigella sativa* oil on micro- and macro-element concentrations using rats as a model system through atomic absorption spectrophotometry. Two experiments were designed, acute for 24 hr and chronic for 60 days. In total, six groups were formulated. Experimental groups were administered 60ml/kg and 120ml/kg of *Nigella sativa* oil. Control groups (0.5 ml distilled water) were run alongside. Animals were dissected toward end of each experiment. Blood was taken directly from the heart and, brain, heart, lungs, stomach, liver, small intestine, kidney, muscle and skin tissues were quickly removed to prepare digests to determine elemental concentrations. Data are presented as mean \pm SEM and comparisons between control and treatment groups were made through one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant difference. Acute administration of *Nigella sativa* at low dose, led to a significant increase of Fe in the blood ($p < 0.001$), Mg in liver, intestine and kidney ($p < 0.001$) and K in brain ($p < 0.001$). Whereas, decrease occurred in Cu concentration in liver and kidney ($p < 0.001$), Mn in blood, skin, lungs, liver, stomach, intestine ($p < 0.001$), Ni in brain, blood, heart, muscle, skin, lungs, liver, stomach and intestine ($p < 0.001$), Na in skin ($p < 0.001$), Ca in brain, blood, heart, muscle, skin, lungs, liver, stomach and kidney ($p < 0.001$), and Cr in all of the organs ($p < 0.001$). The high dose (120ml/kg) showed significant decrease in the Zn, Cu, Cr, Mn, and Ni concentration in all of the organs ($p <$

0.001); Na in blood, skin and lungs ($p < 0.001$), while Ca in skin ($p < 0.001$). In contrast, significant increase occurred in Fe in the liver ($p < 0.001$), Mg in brain and liver ($p < 0.001$), Ca in brain, heart, lungs and intestine ($p < 0.001$), while K increased in all of the organs ($p < 0.001$). Chronic low dose of *Nigella sativa* caused significant increase in Zn in the heart ($p < 0.001$), Cu in stomach and intestine ($p < 0.001$), Mn in intestine ($p < 0.001$), Ni in brain, heart, lungs and kidney ($p < 0.001$), Na in brain, heart, muscle, skin, lungs, stomach and kidney ($p < 0.001$), Mg in brain, blood, heart, muscle, skin, liver, stomach, intestine and kidney ($p < 0.001$), while Ca in brain, heart, lungs, liver, stomach, intestine and kidney tissues ($p < 0.001$). Whereas, significant decrease occurred in the Cu in lungs ($p < 0.001$), Cr in brain, blood, heart, muscle, lungs, liver, stomach and kidney ($p < 0.001$), Ni in muscle, skin, liver, stomach and intestine ($p < 0.001$). The high dose showed significant increase in the Cu in stomach and intestine ($p < 0.001$), Ni in brain, blood, heart, lungs and kidney ($p < 0.001$), K in skin ($p < 0.001$), while Na, Mg, Ca increased in all of the organs ($p < 0.001$). In contrast, significant decrease occurred in Fe concentration of brain, heart, skin, lungs, stomach and intestine ($p < 0.001$), Zn in blood and muscle ($p < 0.001$), Cu in blood, skin and lungs ($p < 0.001$), Mn in brain, blood, muscle, liver and kidney ($p < 0.001$), Ni in muscle, skin, liver, stomach and intestine ($p < 0.001$), while Cr in all of the organs ($p < 0.001$). The current study concludes that the oil of *Nigella sativa* seeds possess modulatory activity on tissue micro- and macro-element concentrations. This necessitates further investigations to understand the underlying physiological mechanism(s).

ACROSOMAL AND DNA INTEGRITY OF SPERMATOZOA FROM PATIENTS VISITING NIH, ISLAMABAD

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Routine semen quality tests are based on sperm motility, concentration and volume or to some extent general morphology. The sperm which appears normal in motility, its acrosome may have become leaky or its DNA may have been damaged that may contribute to infertility but are masked during routine tests. The objective of present study was to determine the relationship between acrosomal integrity, general sperm morphology and DNA integrity with routine semen quality tests in infertile males. For this purpose semen samples from 50 infertile and 10 normal healthy males were collected at Reproductive Physiology Lab, National Institute of Health (NIH), Islamabad. The semen volume, sperm motility and concentration were assessed and samples were fixed and transported to Animal Physiology Laboratory, Department of Zoology, PMAS Arid Agriculture University, Rawalpindi, for evaluation of acrosome intactness, general sperm morphology and DNA integrity. Independent t test was used to compare quality parameters between infertile and normal males, while Pearson correlation was used to study the relationship between different semen quality parameters. Our results showed that all semen parameters vary ($P < 0.05$) among infertile and normal males except semen volume. Further, semen volume, motility and concentration are correlated with one another while morphology, acrosomal integrity and DNA integrity are independent parameters. It is concluded from the present study that evaluation of morphology, acrosome and chromatin damages are also necessary along with other routine parameters.

IMPACT OF CITRIC ACID SUPPLEMENTATION TO *MORINGA OLEIFERA* LEAF MEAL BASED DIETS ON NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE AND HEMATOLOGICAL PARAMETERS OF *LABEO ROHITA* FINGERLINGS

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A feeding trial was carried out to study the impact of citric acid (CA) supplementation to *Moringa oleifera* leaf meal (MOLM) based diets on growth performance, nutrient digestibility and hematological parameters of *Labeo rohita* (*L. rohita*) fingerlings. (MOLM) based diet was subdivided into one control diet (0% CA) and five test diets, each supplemented with 1%, 2%, 3%, 4% and 5% CA, respectively. The fingerlings were fed twice daily for 90 days at the rate of 5% of live wet body weight on their respective diets. The results revealed significant ($P < 0.05$) improvement in overall growth performance and nutrient digestibility of *L. rohita* fingerlings fed CA supplemented diets compared to control diet. During this study, fingerlings fed 3% and 4% CA supplemented diet displayed significantly increased ($P < 0.05$) weight gain (WG), weight gain % (WG%), specific growth rate (SGR) and significantly ($P < 0.05$) decreased FCR compared to all other diets. However highest value of WG (23.73), WG% (255.07), SGR (1.41) and lowest value of FCR (1.28) was observed at 3% CA level. Highest crude protein (67.40%), crude fat (70.51%) and gross energy (65.67%) digestibility was also observed in fingerlings fed 3% CA supplemented MOLM based diet. CA supplementation in MOLM based diet also revealed significant improvement in various hematological parameters (RBCs, WBCs, PLT, Hb, blood PCV, MCHC, MCH and MCV). The study results showed that 3% CA supplementation in MOLM based diet can improve overall fish growth performance, nutrient digestibility and hematological parameters.

INVESTIGATING PAKISTANI COHORT TO DETERMINE THE ROLE OF ANGIOTENSIN CONVERTING ENZYME GENE IN GLAUCOMA

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Glaucoma is one of the leading causes of blindness in the world, with three major types: primary open angle glaucoma (POAG), primary angle closure glaucoma (PACG) and pseudoexfoliative glaucoma (PEXG). Besides other clinical features these different types of glaucoma are primarily characterized by the presence of an increased intra-ocular pressure (IOP), which is under the regulation of the local Renin Angiotensin System. In this system the Angiotensin Converting Enzyme (ACE) plays a central role in the maintenance of IOP in the eye. ACE insertion/deletion (I/D) polymorphism has been implicated in disease susceptibility in various diseases such as Diabetic Nephropathy, Hypertension, Preeclampsia and Ischemic Stroke, however, till date the role of ACE in glaucoma is not clear. Therefore, in the current study the role of ACE

I/D (rs4646994) was investigated in causing POAG, PCAG, and PEXG. This study comprises of genotyping a total of 857 samples among which 126 samples were of PCAG, 264 of POAG, 267 PEXG and 200 samples were healthy controls. A strong association of the ACE I/D genotype frequency distribution was observed with both POAG (chi square (χ^2) = 6.82 [p=0.03], recessive model odds ratio (OR) = 1.66 [95% confidence interval (CI)=1.03- 2.70, p=0.03]) with allele frequencies distribution (χ^2 =1.02 [p=0.31], OR = 1.15 (0.87-1.50) p =0.34) and PEXG (chi square (χ^2)=26.7 [p=<0.001], recessive model odds ratio (OR) =2.86 [95% confidence interval (CI) =1.80-4.56, p=0.001]), which was also significant for the allele frequencies distribution (χ^2 =10.8 [p=0.00], OR=1.55 (1.187-2.02), p=0.00). Consequently, these results indicate the direct involvement of local RAS in the eye with the regulation of aqueous humour and thus maintenance of IOP with the susceptibility of developing POAG and PEXG.

PRODUCTIVE PERFORMANCE, EGG QUALITY AND HATCHING TRAITS OF JAPANESE QUAIL REARED UNDER DIFFERENT LEVELS OF GLYCERIN

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A study evaluated subsequent effect of glycerin on productive performance, egg quality and hatching traits in Japanese quail. A total of 200 birds were arranged according to completely randomized design into 5 treatment groups having 5 replicates of 8 birds each (6 female + 2 Males). Treatment consisted 5 levels of glycerin i.e., 2.5, 5, 7.5 and 10 % and control group. Birds were fed with different levels of glycerin during rearing period of six weeks and their subsequent effect on productive performance, egg quality and hatching traits were observed. Data were collected regarding productive performance for 16 weeks, however, egg quality and hatching traits were recorded during pre-peak (at 4th week), peak (at 12th week) and post peak (at 16th week) phase. Productive performance, egg quality and hatching traits did not differ significantly throughout the experimental period. It is concluded that glycerin can be used as replacement of energy source having no effect on productive and reproductive performance in Japanese quail.

EFFECT ASSESSMENT OF INOCULATION AGAINST IXODID TICKS *HYALOMMA ANATOLICUM ANATOLICUM* (KOCH, 1844) AND *HYALOMMA AEGYPTIUM* (LINN. 1758) USING EXTRACTS FROM ATTACHMENT CONES MADE UP OF CEMENT PROTEINS OF TICKS AS SOURCE OF ANTIGEN

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Experiment was designed to develop vaccine from ticks as anti-tick utilizing partly fed, unfed and fully fed tick cement cone antigen that was demodulated from the two ticks' species *Hyalomma a. anatolicum* (Koch) and *H. aegyptium* (Linnaeus). Ticks were collected from different

cattle farms of district Quetta. For purification of proteins cement cone, methods (Pharmacia) were used under denaturing conditions. Thrice of the phases of purification were captured, intermediate purification and polishing (Amersham-Pharmacia Biotech). Specimens of *Hyalomma a. anatolicum* and *H. aegyptium* were separately placed for 1 to 2 days among ticks which were incompletely fed, unfed and fully fed. In order to dislodge the cement cone from mouth parts of ticks and to take out remaining cement dissecting microscope (Switzerland) was used. For determination of molecular weight of protein, Sodium Dodecyl Sulphate Polyacrylamid Gel Electrophoresis (SDS-PAGE) was acclimating as a conceptual source. Stage reactive and immunogenic cross reactive vaccine development from ticks cement cone used as an antigen of anti-ticks and it is used to check effectiveness through different parameters also to monitor constant acuteness of this vaccine adjoin morphological stages of ticks and as well as to analyze its immunogenicity and added ambit such as stage reactivity, antiserum reactivity and consistent intensity. Using SDS-PAGE and electroblotting the results indicated that positive specific reactions showed dark brown bands and these results. It was recommended that tick larval secretory cement cone of *H. a. anatolicum* and *H. aegyptium* exposed immunogenic, cross reactive, phase reactive and extremely common labeled immuno-positive bands of 23 KDa cement cone proteins.

EFFECT OF PHYTASE AND CITRIC ACID SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE OF *LABEO ROHITA* FINGERLINGS FED ON COTTONSEED MEAL BASED DIETS

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A feeding trial of 70 days was conducted in specially designed tanks to investigate the synergetic effects of phytase and citric acid addition at variable levels on the growth performance and nutrient digestibility of *Labeo rohita* fingerlings fed on cottonseed meal based diets. Nine test diets were prepared by supplementation of phytase (0, 400, 800 FTU kg⁻¹) and CA {0g (0%), 60g (2%), 120g (4%)}. Current research showed that use of phytase and CA in cottonseed meal based diets resulted highest growth performance and nutrient digestibility of *L. rohita* fingerlings. Higher nutrient digestibility (Crude protein 68%, Crude fat 77%, Gross energy 75 kcal g⁻¹) was found at 400 FTU Kg⁻¹ and 120g (4%) of phytase and citric acid supplementation respectively. The maximum weight gain (23g), weight gain% (489%), minimum FCR (1.21) and higher SGR (2.53), values for above mentioned diet showed that fish was in healthy condition as compared to reference and other test diets. The recommended levels of supplementation for phytase and CA are 400 FTU Kg⁻¹ and 120g (4%) respectively for *L. rohita* fingerlings fed on cottonseed meal based diets.

**EFFECTS OF PHYTASE AND CITRIC ACID SUPPLEMENTED CORN GLUTEN (30%)
MEAL BASED DIETS ON THE MINERAL DIGESTIBILITY IN *CIRRHINUS*
MRIGALA FINGERLINGS**

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Phytic acid is a major part of oilseed meals which reduces bioavailability of minerals to fish. Phytase and citric acid supplementation enhances the bioavailability of minerals while on the other hand it reduces the feed cost. The given research project was planned to check the effects of citric acid and phytase supplementation, in corn gluten (30%) meal-based diets, on mineral digestibility of *Cirrhinus mrigala* fingerlings. The diets were formulated to supply adequate levels of all required nutrients for normal fish growth. The chromic oxide was included as an indigestible marker in feed (1%). Triplicate tanks were assigned for each treatment. Fish was fed at the rate of 5% of live wet weight of fish. Effect of each treatment on the minerals digestibility was calculated by standard formulae. It is concluded that addition of 5% citric acid and 500 FTUkg⁻¹ phytase in corn gluten (30%) meal-based diet is most effective among the levels to release the chelated minerals to fish (*C. mrigala*) from phytate complexes. Highest ADC% of minerals (Ca 68%, P 77%, Na 64%, K 62%, Mg 53%, Fe 64%, Cu 68%, Mn 67% and Zn 74%) was observed in the fish fed at above mentioned level. Present results suggest that use of citric acid and phytase improves the fish performance when both were used in corn gluten (30%) meal-based diets.

**DISCLOSURE OF THE HUMAN HEART SECRETS, ITS CONDITION AND
INFLUENCE ON SPEECH AND ACTIONS IN THE LIGHT OF QURAN AND SCIENCE**

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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 and the heart serves as a control room for communication with 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 a 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.

NOVEL APPROACH FOR ZOOLOGIST TO FACE NEW CHALLENGE

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World population is increasing day by day. To feed this population we need to increase crop, meat, milk, egg and fish production. To increase cereal and other crops production, more lands are being used. It squeezed land to produce different animals including livestock and fish. So new technology need to be developed for high yielding varieties of animal using small amount of land. To increase the production of livestock, farmers are using antibiotic, steroid hormone and other drugs which have detrimental effect on human health. Again infected animals are spreading micro-organisms which can cause zoonotic disease to human beings. Food safety is now more important than food security. So researcher need to find out alternative feed additives or drug which are acceptable for human health as well can increase the production. In addition, large number of animals also can cause environmental pollution by producing large amount of methane gas and ammonia nitrogen loss through urine. Poultry farms are producing large ammonia through uric. Researchers need to find out ways to solve these issues. Different herbs, spices and essential oil could be used for these purposes. Large number of population needs large number of industries. In third world countries most of the industries throw their wastes directly to the water. As a result water quality is deteriorating day by day. It has detrimental effect on aquatic animal including fish. Heavy metal toxicity of water body can cause health hazard of human beings through bioaccumulation of these heavy metals in fish. Researchers need to work on it to purify water quality by using organic substances including different micro-organisms. Huge number of population is producing large amount of waste including kitchen, animal, clinical and industrial waste. These wastes are causing environmental pollution, bad odor and other health hazards. But these wastes can be converted in to bio-resource. Researchers can produce biogas, electricity by using these wastes. Different types of enzymes, probiotic, yeast, bacteria and other expected micro-organisms can be produced. That can open a new era for researchers and entrepreneurs who are working with biology.

AVAILABILITY OF MINERALS TO *CATLA CATLA* FINGERLINGS FED PHYTASE SUPPLEMENTED *MORINGA OLEIFERA* LEAF MEAL BASED TEST DIETS

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Present research work was carried out to estimate the influence of phytase supplementation on mineral digestibility of *Catla catla* fingerlings fed *Moringa oleifera* leaf meal (MOLM) based diets. Presence of anti-nutritional factors such as phytate in plant by-products reduces the bioavailability of minerals to fish, resulting in poor fish performance. Moringa by-product such as

Moringa oleifera leaf meal (MOLM) was used as test ingredient to formulate six test diets and were supplemented with phytase levels (0, 300, 600, 900, 1200 and 1500 FTU kg⁻¹). The fingerlings were fed at the rate of 4% of live wet weight twice a day and faeces were collected from each tank. On the basis of results it was noted that phytase supplementation showed significant ($p < 0.05$) improvement in bioavailability of minerals. Maximum minerals digestibility was noted at 900 FTU kg⁻¹ level of phytase supplemented MOLM based test diet. It was concluded that phytase supplementation at 900 FTU kg⁻¹ level is helpful to develop an eco-friendly and cost effective fish feed by using MOLM based diet.

7. TOXICOLOGY

TOXICITY OF ATRAZINE (HERBICIDE) TO BILIRUBIN PROFILE (TOAL BILIRUBIN, DIRECT BILIRUBIN AND INDIRECT BILIRUBIN) OF FRESH WATER FISH, GRASS CARP (*CTENOPHARYNGODON IDELLA*)

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Environmental pollution by toxicants (herbicides) has become one of the most consequential quandaries which has been incremented in the last decades and has magnetized the attention of researchers worldwide and is responsible for contamination of aquatic system. In this regard a study was conducted to demonstrate the sub-lethal effect [acute and chronic toxicity (LC₅₀)] of various concentration of atrazine on bilirubin profile (total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IDB)) of fresh water fish, grass carp (*Ctenopharyngodon idella*). Well acclimated fish were exposed for 01(24), 02(48), 03(72), 04(96), 10(240), 15(360) and 25(600) days (hrs) against different concentration of atrazine like 15, 13, 10, 08, 06, 04 and 02 μL^{-1} respectively. TB and DB was analyzed by biochemical analyzer set (Merck 300 biochemistry analyzer) while IDB was obtained by subtracting DB from TB. Control group concentration for TB, DB and IDB was 0.23, 0.07 and 0.16mmol/l respectively. TB concentration obtained after treatment was 0.6, 0.56, 0.43, 0.4, 0.33, 0.25 and 0.24mmolL⁻¹ respectively. DB concentration was 0.4, 0.35, 0.33, 0.28, 0.26, 0.2, and 0.2 mmolL⁻¹ and IDB concentration was 0.2, 0.21, 0.1, 0.12, 0.07, 0.05 and 0.04mmolL⁻¹ respectively. Results indicate that markedly upturn in concentration of bilirubin profile (denoted by P<0.05 (Significant), P≤0.01(Highly significant) and P≤0.001 (Maximum highly significant)) was observed, indicating hemolysis and damage of hepatocytes.

SPECTROSCOPIC STUDY OF HEAVY METALS IN BABY FOOD AND INFANT FORMULAE OF DIFFERENT BRANDS AVAILBLE IN LAHORE

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In present work concentrations of trace metals; iron, copper, zinc, cobalt, nickel and magnesium, toxic metals; cadmium and lead, micro nutrients; sodium and potassium in twenty seven samples of infant milk-(starter formula and follow up formulae) and baby foods (cereals and milk flavors) were measured. These elements were analyzed by atomic absorption spectrometer prior to acid digestion. The levels of elements in analyzed samples were found to be under legal limits. Nickel was found to be higher in concentration in some infant food that ranges 0.008-0.09mg/g and in infant cereals ranges 0.005-0.01mg/g. The range of investigated metals zinc, iron, copper, cadmium, lead, sodium, potassium and magnesium, is 0.0005-0.12mg/g, 0.002-0.13mg/

0.0005-0.45 mg/g, 0.001-0.04mg/g, 0.001-0.21 mg/g and 0.01-1-2.82mg/g,0.03-9.9 and 0.01-0.046 in infant food and cereals .The estimated intakes of these metals are within recommended tolerable levels of these elements. Protein content is determined through Lowry method that ranges 0.02-2.1mg/0.2g. Moreover infant formulas were analyzed for bacterial load (probiotics) and most of the products analyzed in this study contained a sufficient amount of bacteria in them.

**COMPARATIVE ANALYSIS OF TOXICOLOGICAL IMPINGE OF ATRAZINE
(HERBICIDE) ON STRESS BIOMARKERS; PLASMA GLUCOSE, TOTAL PROTEIN
AND LIPID PROFIE OF FRESH WATER FISH, GRASS CARP
(CTENOPHARYNGODON IDELLA)**

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Present study was experimented for the purpose to contemplate comparative analysis of the acute and chronic toxicity of atrazine on biochemical parameters; Plasma glucose, total protein and lipid profile of freshwater grass carp, (*Ctenopharyngodon idella*). Above 15 μL^{-1} , the LC₅₀ was recorded revealing sensitivity of grass carp to atrazine. Grass carp was exposed to atrazine for 01(15 μL^{-1}) 02(13 μL^{-1}), 03(10 μL^{-1}) and 04(08 μL^{-1}) days/concentration for scrutinizing acute toxicity. Likewise, fish were exposed to atrazine for 10(06 μL^{-1}), 15(04 μL^{-1}) and 25(02 μL^{-1}) days/concentration for scrutinizing chronic toxicity. Fish Blood was analyzed via Merck micro lab 300 biochemistry analyzer. Plasma glucose downturn was observed during acute and chronic toxicity at exposure for 01(15 μL^{-1}) and 10(06 μL^{-1}) respectively while on remaining days/concentrations in both acute and chronic toxicity groups an inclined in glucose concentration was observed. Continuous markedly decline in total protein and lipid profile was observed (denoted by P<0.05, P<0.01 and P<0.001) during acute as well as chronic toxicity, when compared with control group concentration. Therefore plasma glucose downturn in concentration could be attributed to the toxic effect of atrazine on the liver while upturn in concentration was in response of stress to cope with new environmental conditions may be due to glycogen breakdown in the liver which consequently elevates blood glucose level or may be decline in the concentration of the total protein and lipids profile because total protein and lipid profile decline for inclining the plasma glucose concentration. Therefore present study indicating that total protein and lipid profile concentration decline for the purpose to inclined plasma glucose concentration to response the stress imposed by the new environmental conditions. Our study is also purposive to fathom that atrazine is causing agent of acute and chronic health problem to aquatic organisms and induced stress in fish body which revealed pessimistic domination on ecosystem of the aquatic organisms.

DOSE DEPENDENT DNA DAMAGE IN ERYTHROCYTES OF FRESHWATER FISH AFTER CHRONIC EXPOSURE TO PESTICIDE MIXTURE

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Aquatic ecosystems are last receptacle of agricultural and industrial effluents which contain highly toxic contaminants in the form of complex mixtures. Among different contaminants, pesticides are highly toxic due to their long persistence, higher bio-accumulative potential, lipophilic nature and adverse effects on non-target organisms. Keeping in view the toxic nature of pesticides, an experiment was planned to assess the genotoxicity or DNA damage in peripheral blood erythrocytes of fish after exposure to endosulfan and chlorpyrifis mixture. Therefore, comet assay was applied to erythrocytes of freshwater fish, *Ctenopharyngodon idella* under controlled laboratory conditions to assess the DNA damage. In first step, 96-hr LC₅₀ value of pesticide (endosulfan-chlorpyrifos) mixture was estimated for *Ctenopharyngodon idella* in a static system. To check the dose dependent DNA damage, fingerlings of *C. idella* were exposed, separately, to four sub-lethal concentrations of mixture viz. 1/3rd, 1/4th, 1/5th and 1/6th of LC₅₀ along with negative and positive control for the duration of 60-days. Three parameters viz. %age of damaged nuclei, genetic damage index (GDI) and cumulative tail length of comets (CTL) were selected to estimate the DNA damage in fish. After 60 days, comet slides were prepared by taking blood from caudal vein of fish and examined under Epi-Fluorescence microscope for estimation of DNA damage. This mixture gave statistically significant ($p < 0.05$) DNA damage in fish peripheral blood erythrocytes at various exposure concentrations. Dose dependent DNA damage in terms of %age of damaged nuclei and GDI were observed, with highest damage at 1/3rd of LC₅₀ as compared to control groups. Incidence of CTL was also observed higher due to 1/3rd of LC₅₀, as evident from their mean value $856.23 \pm 0.09 \mu\text{m}$ while it was significantly lower ($3.38 \pm 0.06 \mu\text{m}$) due to negative control exhibiting dose dependent DNA damage. Damage induced by this mixture suggested a serious concern towards their potential danger to the survival of freshwater fish in natural environment of Pakistan. This study also confirmed that the comet assay is a useful technique for assessing the DNA damage in fish.

IN VIVO STUDY OF EXTRACTS OF ARTEMISIA VULGARIS AGAINST HEPATOCELLULAR CARCINOMA INDUCED BY DIETHYLNITROSOAMINESHAUKAT ALI*, MUBASHIR EJAZ, KAMRAN KHURSHID DAR, SUNDAS NASREEN,
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Diethylnitrosoamine (DEN) is a carcinogenic compound and used to induce cancer and tumor in mice. Liver damage caused by DEN generally reflects instability of liver cell metabolism which leads to distinctive changes in the serum enzyme activities. Plants are a source of phytochemical compounds and secondary metabolites that play a major role in their medicinal properties. The present study was designed to investigate the effect of DEN on the biomarkers of liver and the anti-cancerous effect of *Artemisia vulgaris* in male Balb C mice. The mice were divided into three groups, 1st received saline solution (control), 2nd received DEN for eight weeks and then extract of leaves of

Artemisia vulgaris for further eight weeks, while 3rd received leaves of *Artemisia vulgaris* extract (LAV) only for sixteen weeks. Hepatocellular carcinoma tumor markers including alanine transaminase, aspartate transaminase, lactate dehydrogenase, gamma glutamyl transpeptidase, 5'-nucleotidase, bilirubin, glucose 6-phosphate dehydrogenase, alpha-fetoprotein and albumin were detected by using specific biochemical analysis kits. Intraperitoneal administration of diethylnitrosoamine (3.5 µl/mg once in a week for eight consecutive weeks in mice) caused significantly increased in levels of alanine transaminase (control: 29.0±1.8 U/L; DEN: 141.5±2.2 U/L), aspartate transaminase (control: 87.9±2.7 U/L; DEN: 474.0±7.6 U/L), lactate dehydrogenase (control: 373.2±15.0 U/L; DEN: 2165.7±78.9 U/L), gamma glutamyl transpeptidase (control: 38.2±1.6 U/L; DEN 146.8±2.6 U/L), 5'-nucleotidase (control: 5.3±0.3 U/L; DEN: 16.3±0.7 U/L), bilirubin (control: 0.9±0.1 mg/dl; DEN: 2.9±0.2 mg/dl), glucose 6-phosphate dehydrogenase (control: 4.1±0.3 mU/ml; DEN: 13.7±0.4 mU/ml) and alpha fetoprotein (control: 13.5±0.9 ng/ml; DEN: 63.4±2.4 ng/ml) was observed. Investigated level of albumin was decreased (control: 6.2±0.4 U/L; DEN: 2.8±0.2 U/L) by intraperitoneal administration of diethylnitrosoamine (DEN). There were no significant changes in levels of biological markers between control and extract of *Artemisia vulgaris* group. Extract of leaves of *Artemisia vulgaris* extract (LAV) in hydromethanolic solvent given by intraperitoneal injection (150 mg/kg once in a week for eight consecutive weeks in mice). The results revealed that *Artemisia vulgaris* had decreased the levels of ALAT (DEN: 141.5±2.2 U/L; LAV: 35.5±1.7 U/L), ASAT (DEN: 474.0±7.6 U/L; LAV: 104.1±1.7 U/L), LDH (DEN: 2165.7±78.9 U/L; LAV: 475.6±16.7 U/L), AFP (DEN: 63.4±2.4 ng/ml; LAV: 19.5±1.0 ng/ml), γ-GT (DEN: 146.8±2.6 U/L; LAV: 45.0±3.0 U/L), 5'NT (DEN: 16.3±0.7 U/L; LAV: 5.5±0.3U/L), Bilirubin (DEN: 2.9±0.2 mg/dl; LAV: 1.1±0.1 mg/dl), G6PDH (DEN: 13.7±0.4 mU/ml; LAV: 5.3±0.4 mU/ml) in DEN treated Balb C mice. With respect to increase the hepato-carcinogenic signals i.e. (ALAT, ASAT, 5'NT, AFP, Bilirubin, γ-GT, G6PDH and LDH), albumin decreased. Therefore, the extract of leaves of *Artemisia vulgaris* has anti-cancerous properties.

INDUCTION OF HEPATOCELLULAR CARCINOMA BY DIETHYLNITROSOAMINE AND PHARMACOLOGICAL INTERVENTION IN BALB C MICE

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Diethylnitrosoamine (DEN) is a carcinogenic compound and used to induce cancer and tumor in mice. Liver damage caused by DEN generally reflects instability of liver cell metabolism which leads to distinctive changes in the serum enzyme activities. The present study was designed to investigate the effect of DEN on the biomarkers of liver and the anti-cancerous effect of *Bergenia ciliata* and *Artemisia vulgaris* in Male Balb c mice. Hepatocellular carcinoma tumor markers including alanine transaminase, gamma glutamyl transferase, lactate dehydrogenase, alpha-fetoprotein, 5'-nucleotidase, aspartate transaminase, albumin, bilirubin and glucose 6 phosphate dehydrogenase were detected by using specific biochemical analysis kits. Diethylnitrosoamine given by intraperitoneal injection (3.5 µl/mg once in a week for eight consecutive weeks in mice) had induced significant changes in levels of biological markers. There were significant changes in levels of ALAT (control: 29.0±1.8 U/L; DEN: DEN: 141.5±2.2 U/L), ASAT (control: 87.9±2.7 U/L; DEN: 474.0±7.6 U/L), LDH (control: 373.2±15.0 U/L; DEN: 2165.7±78.9 U/L), AFP (control: 475.6±16.7

ng/ml; DEN: 63.4±2.4 ng/ml), G6PDH (control: 4.1±0.3 mU/ml; DEN: 13.7±0.4 mU/ml), γ -GT (control: 38.2±1.6 U/L; DEN: 146.8±2.6 U/L), 5' NT (control: 5.3±0.3 U/L; DEN: 16.3±0.7 U/L), bilirubin (control: 0.9±0.1 mg/dl; DEN: 2.9±0.2 mg/dl) and albumin (control: 6.2±0.4 U/L; DEN: 2.8±0.2 U/L) of control and DEN group. Extract of rhizome of *Bergenia ciliata* (RBC) and leaves of *Artemisia vulgaris* in hydro-methanolic solvent given by intraperitoneal injection (150 mg/kg once in a week for eight consecutive weeks in mice) did not induce significant changes in levels of biological markers. There were no significant changes of ASAT (control: 29.0±1.8 U/L; RBC: 37.5±3.0 U/L; LAV: 37.5±1.7 U/L), ALAT (control: 29.0±1.8 U/L; RBC: 37.5±3.0 U/L; LAV: 37.5±1.7 U/L), LDH (control: 373.2±15.0 U/L; RBC: 511.9±28.6 U/L; LAV: 475.6±16.7 U/L), AFP (control: 475.6±16.7 ng/ml; RBC: 16.0±1.3 ng/ml; LAV: 19.5±1.0 ng/ml), γ -GT (control: 38.2±1.6 U/L; RBC: 43.7±2.1 U/L; LAV: 45.0±3.0 U/L), 5' NT (control: 5.3±0.3 U/L; RBC: 4.9±0.2 U/L; LAV: 5.5±0.3 U/L) and bilirubin (control: 0.9±0.1 mg/dl; RBC: 1.2±0.1 mg/dl; LAV: 1.1±0.1 mg/dl), albumin (control: 6.2±0.4 U/L; RBC: 6.1±0.4 U/L; LAV: 5.7±0.3 U/L), G6PDH (control: 4.1±0.3 mU/ml; RBC: 4.0±4.0 mU/ml; LAV: 5.3±0.4 mU/ml) of control and *B. ciliata* and *A. vulgaris* mice groups. It was concluded from the current studies that given concentration of DEN for eight weeks had induced HCC in mice while the treatment of extract of rhizome of *Bergenia ciliata* and leaves *Artemisia vulgaris* had attenuated the effects of DEN in Balb C mice. Therefore, the extract of rhizome of *Bergenia ciliata* leaves *Artemisia vulgaris* have potential anti-cancerous activity.

**DETERMINATION OF TOXICITY OF DIMLIN AGAINST THE PISTOL SHRIMP
*ALPHEUS INOPINATUS***

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Dilmin or diflubenuron is the pesticide used against domestic, agricultural and medically important pests for their control in the laboratory and in the field as well. The application of this compound is generally in the aquatic areas which may contain insect larvae but also crustaceans, fishes etc. In the present study the toxic effect of dimilin was evaluated against a caridean-the pistol shrimp *Alpheus inopinatus*. The shrimps were collected from the Karachi coast. The potency of the test compound as a toxic substance was judged by observations on mortality of the shrimp, which shows 13-87%, when treated with dimilin at 200-600 ppm doses after 24 hours. The dosage –mortality curve (LC50) was drawn and LC 50 was found to be 380ppm. This study may help in undertaking further studies on development rate pattern of protein and biometric studies of the shrimp.

**SCRUTINIZING ACUTE AND CHRONIC TOXICOLOGICAL IMPINGE OF ATRAZINE
(1-CHLORO-3-ETHYLAMINO-5-ISOPROPYLAMINO-2, 4, 6-TRIAZINE) ON AQUATIC
ORGANISMS THROUGH EVALUATING STRESS BIOMARKER; PLASMA GLUCOSE
CONCENTRATION OF FRESH WATER FISH; GRASS CARP,
(*CTENOPHARYNGODON IDELLA*)**

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Herbicide praxis in agricultural fields to curb herbs is utterly toxic to non target areas like aquatic bodies and impinge aquatic organism distinctively fish health through impairment of

metabolism, sometimes leading to mortality. So for contemplation of acute (short term) and chronic (long term) toxicological effects of atrazine, study was designed to determine the potential detrimental effects of commercial utilization of chemical named; atrazine, on aquatic organisms through evaluating stress biomarker; plasma glucose (energy currency) of freshwater fish; grass carp, (*Ctenopharyngodon idella*) for 01(24), 02(48), 03(72), 04(96), 10(240), 15(360) and 15(600) days(hrs) under the dose 15µl/L, 13µl/L, 10µl/L and 08µl/L, 06µl/L, 04µl/L, 02µl/L respectively. Above 15µl/L LC50 was recorded indicating grass carp sensitivity to atrazine. Control group concentration of plasma glucose was 42.3mmol/L. Paramount downturn during acute toxicity and chronic toxicity was observed after 24hrs (15µl/L) and 240hrs (06µl/L) exposure respectively among treated groups while in both groups with the declining of dose quantity an inclined in glucose concentration was observed. Downturn in concentration could be attributed to the toxic effect of atrazine on the liver and upturn in concentration may be due to inhibition of the gluconeogenesis process which enhances glycogen breakdown in the liver and consequently elevates blood glucose level in response of stress to cope with new environmental conditions. Our study is also purposive to fathom that atrazine is causing agent of acute and chronic health problem to aquatic organisms and induced stress in fish body which revealed pessimistic domination on ecosystem of the aquatic organisms.

EVALUATING THE HEAVY METALS CONCENTRATIONS; LEAD AND CADMIUM IN DIFFERENT ORGANS OF TORKAY (*SCHIZOTHORAX PLAGIOSTOMUS*) OF INDUS RIVER COLLECTED FROM KUND, JEHANGIRA AND ALA DAIR, DISTRICT SWABI, KPK-PAKISTAN

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Objectives of the present study was to evaluate the heavy metals; lead and cadmium concentration in different tissues (Gills, Swim Bladder and Muscles) of Torkay (*Schizothorax plagiostomus*) collected from Indus River at Kund, Jehangira and Ala Dair, District Swabi, Khyber Pakhtunkhwa, Pakistan. Present study was conducted for three months; Feb-April 2016. During the present study it was observed that in February, concentration of cadmium were maximum in gill tissue (0.03 gm) followed by swim bladder tissue (0.02 gm) and muscle tissue (0.01 gm) while lead were not detected in any tissue. Similarly in March, concentration of cadmium were maximum in gill tissue (0.05 gm) followed by swim bladder tissue (0.40 gm) and muscle (N.D). While lead were maximum in gill tissue (0.02 gm) followed by swim bladder tissue (0.01 gm) and muscle tissue (N.D). Likewise in April, concentration of cadmium were maximum in gill tissue (0.04 gm) followed by muscle tissue (0.03 gm) and swim bladder (N.D). While lead concentration were maximum in gill tissue (0.06 gm) followed by swim bladder tissue (0.02 gm) and muscle tissue (0.01 gm). Cd and Pb were maximum accumulated in gill tissues (Cd = 0.03gm, 0.05gm and 0.04gm while Pb=0.00gm, 0.02gm and 0.06gm) followed by swim bladder (Cd= 0.02gm, 0.40gm, and 0.00gm while Pb= 0.00gm, 0.01gm, 0.02gm) and Muscles tissues (Cd= 0.01gm, 0.00gm and 0.03gm while Pb = 0.00gm, 0.00gm and 0.01gm).Cd was maximum accumulated in gills tissues in March followed by April and February while Pb was maximum accumulated in April followed by March and February. Likewise Cd was maximum accumulated in swim bladder in March followed by February and April while Pb was maximum accumulated in April followed by March and February. Similarly Cd was maximum accumulated in Muscles tissue in April followed by the

February and March while Pd was found to maximum accumulated in April followed by March and February. Beside this it was also observed that both the Cd and Pb concentration was found to be maximum in gills, swim bladder and Muscles tissues which were collected from Ala Dair followed by Jahangera and Kund. Therefore the present result indicates that Indus River at these three sites have concentration of toxic metal that are Cd and Pb and such metals also accumulate in the tissue of fishes and the concentration observed in the present study when compared with permissible limits reported by WHO (World Health Organization) so it was observed that the accumulation yet did not reach to the toxic level and have no adverse effect on species as well as on the human when they ingest via food chain consumption but if the additions of such metal is continued from the external environment so it will have negative impact on the aquatic fauna as well as on human being.

BIOACCUMULATION OF PESTICIDE RESIDUES IN DIFFERENT TISSUES OF WALLAGO ATTU SAMPLED FROM AGRICULTURALLY IMPACTED SEGMENT OF THE RAVI, PAKISTAN

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We conducted a study to investigate the accumulation of pesticides in different tissues of *Wallago attu*, sampled from Balloki Headworks of the Ravi, Pakistan. The study provides baseline data on accumulation of organochlorine pesticides (OCPs) and other pesticide residues in gills and muscles of the fish. Results of the present study depicted the accumulation of high concentrations of pesticide residues in both the fish muscles and gills, however, muscles were found highly contaminated as compared to gills. The overall order of pesticides' accumulation in fish gills and muscles appeared as DDT > DDE > carbofuran > endosulfan. The findings of this study will be helpful in minimize the significant use of pesticides and to evaluate the contribution of Pakistan towards persistent organic pollutant (POPs) emissions in global environment and effectiveness of control measures taken by regional and international authorities under Stockholm convention in Pakistan.

BIOACCUMULATION OF METAL IONS IN DIFFERENT TISSUES OF WALLAGO ATTU SAMPLED FROM ANTHROPOGENICALLY IMPACTED SEGMENT OF THE RAVI, PAKISTAN

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A study was conducted to investigate the accumulation of metal ions in different tissues of *Wallago attu*, sampled from Balloki Headworks of the Ravi, Pakistan. We examined the

accumulation of Zn, Cr and Cu in heart, liver and skeletal muscles of the sampled fish. Results of the present study revealed that the accumulation of metals fluctuated significantly in different organs of *Wallago attu*. The accumulation of Cr was found significantly higher as compared to Zn and Cu. The overall order of metal accumulation in different tissues of *Wallago attu* was Cr > Zn > Cu. However, the order of metal burden in different organs of the fish appeared as heart > muscle > liver. Our findings of the present investigation indicated that heavy discharge of enormous metallic compounds has adversely affected the freshwater habitats and still the level of metal pollution is going to increase rapidly as a result of various anthropogenic activities. So, suitable steps must be taken in order to control increasing level of metal pollution.

CHROMIUM AND LEAD ACCUMULATION IN DIFFERENT ORGANS OF *LABEO ROHITA*

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The aim of this study was to determine the bioaccumulation of heavy metals like chromium (Cr) and lead (Pb) in the organs/tissues of rahu (*Labeo rohita*) from Head Taunsa and Head Punjnad, Punjab, Pakistan. The analysis was carried out using atomic absorption spectrophotometer. Significant ($p < 0.05$) difference was observed in the heavy metal concentrations among the liver, muscle, skin and gills in this fish species. Chromium concentration in muscle (0.725 ± 0.021) and skin (0.730 ± 0.014) were observed significantly ($P \leq 0.05$) difference between Taunsa than head Punjnad while others organs i.e. kidney, gills and liver remained non-significant ($P \leq 0.05$). Lead concentration in liver were statically significantly ($P \leq 0.05$) higher (1.550 ± 0.084) at Taunsa than head Punjnad while others remained non-significant ($P \leq 0.05$).

EFFECT OF $AlCl_3$ MEDIATED TOXICITY ON THE HEMATOBIOCHEMICAL PROFILE OF ADULT MALE ALBINO MICE

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The aim of this study was to report the effects of $AlCl_3$ mediated toxicity on the hematobiochemical profile of male albino mice. Eight week old male albino mice were used as experimental animals and were divided into two groups. First group was treated with 80mg/Kg body weight of $AlCl_3$ for 16 days while control group was treated with saline solution for the same period of time. Complete blood count (Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, packed cell volume, hemoglobin level, total red and white blood cell count, total lymphocytes, monocytes, neutrophil count and total platelets count)

and selected serological parameters [Cholesterol, Alanine transaminase (ALT), Aspartate transaminase (AST), total protein, Creatinine and triglycerides] were determined in both experimental treatments at the end of dose supplementation. Our results revealed that oral supplementation of 80mg/Kg body weight of AlCl₃ for 16 days did not affect ($P > 0.05$) any of the studied parameters of complete blood count. On the other hand, serological parameters like triglycerides ($P = 0.0075$), total proteins ($P = 0.042$) and creatinine ($P = 0.0038$) were significantly higher in treated male mice when compared with their untreated control group indicating the hazardous effects of AlCl₃ on blood chemistry of adult male albino mice..

EFFECT OF BIFENTHRIN CONTAMINATED FEED ON GROWTH PERFORMANCE, ORGAN WEIGHT AND HEAMATOLOGICAL INDICES IN BIRDS (*COLUMBA LIVIA*)

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Birds meat is a high quality source of proteins and it is one of the most important sector of poultry and livestock industry. Despite its importance widespread use of pesticides is a major constraint in bird's population and productivity. The present study was aimed to evaluate the sub chronic toxic effects of Pyrethroid (Bifenthrin) insecticide on growth performance, organs weight and some hematological indices in birds. A total of 24 pigeon squabs (*Columba livia domestica*) about six weeks old were used and randomly divided into four equal groups allocated three treatment groups (B-C) and one control (A). On the basis of LD₅₀ toxicity factor, bifenthrin insecticide contaminated feed with a concentration of 40 mg/kg, 60 mg/kg and 80 mg/kg were given to groups B-C respectively for consecutive 8 weeks while group A birds received without bifenthrin contaminated feed. Birds of all groups (A-D) served same quantity of grains (*ad libitum*) diet/day during entire period of study. Clinical signs diarrhea, excessive salivation, lethargy, convulsion and tremors were observed in treated birds. A significant difference ($P < 0.05$) in feed intake, body weight gain and final body weights in birds of all groups were documented. Liver kidneys, testes and ovaries weights were observed significantly ($P < 0.05$) different in treatment groups C and D in comparison with control and treatment group B. Heamatological indices (Hb, RBC, WBC, PCV and PLT) were found significantly ($P < 0.05$) decreased in birds of all treatment groups as compared to control. Therefore, it is concluded that bifenthrin contaminated feed could affect the growth performance, organ weight and heamatological characteristics.

INVESTIGATIONS ON WATER-BORNE ACUTE TOXICITY OF MN+AS MIXTURE TO THE FISH, *LABEO ROHITA*

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In order to determine the acute toxicity of Mn+As mixture for *Labeo rohita*, in terms of 96-hr LC₅₀ and lethal concentration, fingerlings of *Labeo rohita* were exposed to water-borne binary

mixture of manganese and arsenic at different concentrations. The experiment was performed under static bioassay at constant water temperature, pH and total hardness of 28°C, 7.4 and 230mgL⁻¹, respectively. During 96-hr exposure period fish were exposed to various concentration of metals mixture and their mortality data were collected against each concentration and analyzed by means of Probit analysis method and median lethal (96-hr LC₅₀) and lethal concentration were computed. Various physico-chemical variables of water medium were also monitored and recorded at 12-hr intervals during each exposure concentration. Relationships among different parameters under study were found out by using correlation analysis. The present study revealed significant differences between 96-hr LC₅₀ and lethal concentration values of Mn+As mixture for *Labeo rohita*. The mean 96-hr LC₅₀ and lethal concentration of Mn+As metals mixture for the fish were determined as 58.78±2.54 and 97.34±2.66mgL⁻¹, respectively. Fish mortality percentage was directly proportional to the exposed concentration of metals mixture. Total ammonia showed highly significant and positive correlations with sodium, potassium, calcium and magnesium of *Labeo rohita* test media. The relationships of dissolved oxygen with total ammonia, sodium, potassium, calcium and magnesium were inversely significant at p<0.01. Carbon dioxide showed negative but highly significant correlation with dissolved oxygen but its relationship with total ammonia, sodium, potassium, calcium and magnesium were positive and highly significant. There existed direct and highly significant relationships of electrical conductivity with the rest of physico-chemical parameters except for dissolved oxygen to which it was highly significant but inversely correlated.

STUDY ON IMPACT OF BIOPESTICIDES ON MORPHOLOGY AND HEMATOLOGY OF COMMON MYNA (*ACRIDOTHERES TRISTIS*)

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The proposed study was conducted for evaluating the effect of biopesticides (which are obtained by the Ethanolic extraction from *Datura stramonium* and *Eucalyptus camaldulensis*) on the morphology, hematology of *Acridotheres tristis* under laboratory conditions of 30±2°C in the Pest control lab of the Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad. The plant extract formulation was prepared by drying the collected leaves and then extracting the active ingredient by using 70% ethyl alcohol with the help of proper apparatus. Then the prepared dose was given as 4000mg/kg body weight of the birds, in the form of pellets for one month. The experiment was repeated for three times. The data regarding the morphological changes was recorded on daily basis. Skin texture and colour changes were observed. Brown color faint out and texture became hard. Some of the hematological parameters such as white blood cells count (WBC's), red blood cells count (RBC's), mean corpuscular hemoglobin (MCH), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb) and mean corpuscular volume (MCV) and showed highly significant results (P<0.01). Feed, water and body weight were also observed and showed highly significant results (P<0.01).

**LYCOPENE SUPPLEMENTATION REDUCES CADMIUM TOXICITY AND
OXIDATIVE STRESS IN POULTRY**

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Cadmium, a toxic heavy metal is being released in large amount into the environment by anthropogenic activity. Poultry is highly susceptible to cadmium toxicity. Lycopene is an antioxidant substance which protects from oxidative insult. The present study was designed to evaluate the protective role of Lycopene on poultry against cadmium. Eighty one day old broiler chickens were used for study. First group served as control. The second group was given cadmium in drinking water at a concentration of 30mg/L. Third group served as a positive control and provided oral dose of lycopene (10mg/kg). In fourth group, Lycopene (10mg/kg) was co-administered orally with cadmium (30mg/L). All the treatments were carried out for 8 weeks. Cadmium treatment resulted in significant ($p<0.05$) increase in ALT, AST, urea and creatinine. Cadmium administration also induced significant ($p<0.05$) increase in the thiobarbituric acid reactive substance, whereas catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GSR) activities were significantly ($p<0.05$) decreased in liver and kidney. Lycopene supplementation significantly ($p<0.05$) ameliorated the levels of ALT, AST, urea and creatinine. Additionally, lycopene administration restored antioxidant defense system and decreased lipid peroxidation significantly ($p<0.05$). In conclusion, oral administration of lycopene prevented the broiler chicks from cadmium induced toxicity.

**THE EFFECT OF AQUATIC POLLUTANTS ON THE CATLA CATLA HEPATIC
GLUTATHIONE S TRANSFERASE**

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On the exposure of pollutants, reactive oxygen species could be generated by oxidative stress in organisms. Glutathione S transferase (GST) is an important biotransformation enzyme family that found in all the organisms that overcome the influences of ROS. This enzyme catalyzes the glutathione to a great variety of their xenobiotic compounds. The objective of my research work was to purify the glutathione S-transferase (GST) enzyme from the liver of fresh water fish *Catla catla*. Fish was collected from natural site i.e. Head Baloki as well as Proka Fish Farm, Faisalabad, Punjab, Pakistan. After the collection, fish samples were dissected to remove the liver and stored it at 4°C. The fish liver was subjected to purification and characterization for GST. Purification was performed by ammonium sulphate and ion exchange chromatography technique. Characterization was done by using different pH of buffer, temperature and CDNB (1-chloro-2,4-dinitrobenzene) substrate concentration. The data obtained from this experiment were statistically analyzed by using microcomputer software to check the differences among different parameters. Significant difference ($p<0.01$) were formed between pond (Proka Fish Farm) and natural fish (Head Baloki).

The inferences of the present study was indicated higher (80.39 UmL⁻¹) hepatic glutathione S transferase activity in fish collected from natural location as compared with pond fish (49.14 UmL⁻¹). Highest GST activity was observed at 6 pH i.e. 57 UmL⁻¹ and 78 UmL⁻¹ for pond and natural site fish respectively. Comparison of means on hepatic GST activity for treatments (Pond and River) at various temperatures revealed as 43.57±2.88 UmL⁻¹ and 66.00 ± 3.30 UmL⁻¹ respectively. This indicated that the Head Baloki (river Ravi) site was more polluted when compared with Proka Fish Farm.

**DETERMINATION OF HEAVY METALS CONCENTRATION IN THE TISSUES OF
SCHIZOTHORAX PLAGIOSTOMUS (HECKEL, 1838) IN RIVER SWAT**

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The current study was conducted in river swat at selected sites such as Charbagh, Odigram and Landakai of district swat. The study was aimed to analyze the bioaccumulation pattern in the tissues such as muscles and gills of freshwater fish *Schizothorax plagiostomus* at the selected sites. *Schizothorax plagiostomus* is economical fresh water belong to class actinopterygii, also known as snow trout. The fish is selected for study because of their nutritive, commercial and consumption values. The samples were collected in triplicate form and the extracted tissues were dissolved by the use of per- chloric and nitric acid along with hotplate for the further dissolution of tissues. The heavy metals like Zinc, lead, chromium and Nickel were determined using Perkin Elmer 2380 atomic absorption spectrophotometer. A great variation occurred in the metals content related to tissue type and sampling sites. High concentration of bioaccumulation was reported at Charbagh site and least occurred at Odigram site. The order of accumulation were Charbagh>Landakai>Odigram. In the same way Cr was the most accumulated heavy metal followed by lead, nickel and Zinc. The order of accumulation were Cr>Pb>Ni>Zn. The concentration was taken in mean ± standard error. Similarly the order of accumulation of metals at Odigram, Charbagh and Landakai were Cr>Ni>Zn>Pb, Cr>Pb>Ni>Zn and Cr>Pb>Ni>Zn respectively and the mean concentration of the heavy metals such as Zn, Pb, Cr and Ni in the muscles at Odigram site were 0.55, 0.21, 1.60 and 0.92ppm respectively and that in gills were 0.42, 0.60, 1.43 and 0.45ppm respectively. Whereas concentration in the muscle at Charbagh site was 0.13, 1.94, 2.18 and 0.51ppm and in gill was 0.25, 0.04, 2.98 and 0.95ppm respectively. Similarly the mean concentrations in the muscle at Landakai site was 0.08, 0.90, 1.32 and 1.71ppm and that in gill was 0.23, 1.50, 1.76 and 0.94ppm respectively. Data demonstrated that heavy metal levels in the tissues of *Schizothorax plagiostomus* were within permissible limits provided by WHO and thus suitable for human consumption but it could be a matter of concern in the near future due to too much industrialization and other activities.

**EVALUATION OF TOXICOLOGICAL EFFECT OF LEAD ACETATE
ON LUNGS OF BROILER CHICKS**AZIA IBRAHIM,¹ MUHAMMAD SHAHBAZ¹ AND ARSHAD JAVID²¹*Department of Zoology, Women University, Azad Jammu and Kashmir, Bagh*²*Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore.*

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Lead exposure is an important public health problem, especially in the urban environment (Tong *et al.*, 2000) and even a low-dose is hazardous (Grandjean, 2010). Lead contaminated dust and lead based paints are the main sources of lead poisoning. However, there are many other sources, including: ceramic glazes, electronic waste, cosmetics, toys, water pipes, solder in canned food and lead from soils (Chaturkova *et al.*, 2010). Clinical and science studies have suggested that lead is devastating to the human body. Lead poisoning accounts for about 0.6% of the global burden of disease (Organization, 2009). Lead enters the human body from the environment by inhalation and through the digestive system. Although human lead toxicity has decreased since discontinuation of the use of lead as a gasoline additive, its exposure continues to be a public health problem across the world (Brody *et al.*, 1994). A number of recent studies confirmed the possible involvement of reactive oxygen species in lead induced toxicity (Upadhyay *et al.*, 2009). Oxidative stress has been implicated for its contribution to lead-associated tissue injury in the lungs, liver, kidneys, brain, and other organs.

**DETERMINATION OF 96-HR LC₅₀ AND LETHAL RESPONSES OF WALLAGO ATTU
TO WATER-BORNE CHROMIUM**

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Water pollution by heavy metals, especially chromium pollution from industrial source can affect aquatic life, all ecosystems and human health directly or through food chain. The present research work was conducted under laboratory conditions to determine 96-hr LC₅₀ and lethal responses of a fish, *Wallago attu*, to water-borne chromium. The fingerlings of fish were kept in glass aquaria having 35-L water capacity. The toxicity tests were conducted at constant total hardness (250mgL⁻¹), water temperature (28 °C) and pH (8) with three replications for each test concentration, separately. During 96-hr acute toxicity trials, the observations on fish mortality were made after every 2 hours. Physico-chemical parameters of water viz. total hardness, temperature, pH, carbon dioxide, dissolved oxygen, total ammonia, sodium and potassium were monitored twice a day during each 96-hr trial. The 96 hour LC₅₀ and lethal concentrations of chromium, with 95% confidence interval, were computed by using the Probit analysis method. Regression/correlation analyses were also performed to find-out relationships among different variables under study. This investigation revealed significant differences between 96-hr LC₅₀ and lethal concentrations of chromium for *Wallago attu*. The mean 96-hr LC₅₀ and lethal concentrations of chromium for this fish were computed as 59.17±1.45 and 90.65±1.09mgL⁻¹, respectively. The chromium concentrations in the test media exhibited statistically highly significant correlation with calcium, sodium and potassium while it showed highly significant but inverse relationship with magnesium contents of the test media. Carbon dioxide showed significantly direct relationship with total

ammonia while it exhibited highly significant but inverse correlation with dissolved oxygen. The dissolved oxygen revealed negatively significant relationship with total ammonia. Calcium exhibited statistically highly significant but negative correlation with magnesium contents of the test media.

TOXIC EFFECTS OF ALUMINIUM TO THE CARNIVOROUS FISH SPECIES

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The metallic ion pollutants are released continuously from both natural as well as anthropogenic sources cause serious hazards to the carnivorous fish species in freshwater ecosystems of Pakistan. Therefore, acute toxicity tests of aluminium, in terms of 96-h LC₅₀ and lethal concentrations, were conducted to evaluate the sensitivity of carnivorous fish species viz. *Channa marulius*, *Mystus seenghala* and *Wallago attu*. The tests were performed under constant water temperature (28°C), pH (8) and total hardness (250mgL⁻¹). The fingerlings of 150mm were first acclimatized to the laboratory conditions for a period of ten days and then transferred to the glass aquaria for toxicity experiments. During the whole acute toxicity trials, fish mortality was observed at two hour interval while various physicochemical variables of water were determined at 12-h interval. Fish mortality data were analyzed through Probit analysis method with 95% confidence interval to estimate 96-h LC₅₀ and lethal concentrations of aluminium for each species. Among the three fish species, *M. seenghala* showed significantly ($p < 0.01$) higher sensitivity towards aluminium with the mean 96-h LC₅₀ value of $62.34 \pm 3.08 \text{mgL}^{-1}$, followed by that of *W. attu* and *C. marulius* for which the same was computed as 86.97 ± 2.72 and $138.20 \pm 3.46 \text{mgL}^{-1}$, respectively. However, the median lethal concentrations of aluminium to the three fish species viz. *C. marulius*, *M. seenghala* and *W. attu* were calculated as 193.85 ± 8.35 , 105.93 ± 6.67 and $123.54 \pm 5.77 \text{mgL}^{-1}$, respectively.

CHEMICAL COMPOSITION AND GROWTH PERFORMANCE OF *LABEO ROHITA* UNDER THE INFLUENCE OF CHROMIUM CHLORIDE HEXAHYDRATE

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To investigate the role of chromium chloride hexahydrate and corn (Gelatinized and Non-Gelatinized) on growth and body composition in *Labeo rohita*, a 120 days trial was carried out. Six experimental diets were prepared by using various levels of chromium chloride hexahydrate (CrCl₃.6H₂O) in diet at the rates of 0.0/G (T1), 0.0/NG(T2), 0.3/G(T3), 0.3/NG (T4), 0.5/G (T5) and 0.5/NG (T6) mg/Kg. Fifty fingerlings were distributed randomly in glass aquaria having dimensions 90LX30WX45H(cm) with 29 L water capacity with two replicates. Results showed that maximum value of total body weight and total body length were recorded in group T3. For body composition maximum deposition of ash was observed in experimental group T6 and dry matter in

group T3. While, highest values of crude protein, crude fat and gross energy were observed in group T5. From results it was concluded that inclusion of chromium in diet with gelatinized corn improved not only growth performance but also increased the deposition of different nutrients in body meat of *Labeo rohita*.

HEMATOLOGICAL RESPONSE OF *CATLA CATLA* TO THE Zn+Ni TOXICITY

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Large quantities of heavy metals into the water bodies released from industrial, domestic and other manmade activities causes water pollution and has become a serious problem in this century. The purpose of the present research work was to monitor the changes in blood profile of *Catla catla* kept under Zn+Ni metal mixture stress. To achieve the goal of this study, proposed fish samples were collected from the local public fish seed hatchery and acclimatized to the laboratory conditions. After acclimatization, the fish was divided into experimental and control group (15 fishes in each group) and experiment was conducted for a period of 2 weeks. Fish was exposed to sub-lethal concentration of Zn+Ni metal mixture. At the end of the experimental trail, blood samples were collected from the experimental and control group. Various hematological parameters WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, RDW-CV, PDW, MPV, P-LCR and including PCT were determined by standard methods. Statistical analysis was done on the data collected in the present study by using ANOVA and measured significant difference ($p \leq 0.05$) between metal treated and control group hematological parameters. The inferences of the present study revealed decrease in most of the hematological parameters in the Zn+Ni metal mixture stressed *C. catla* in comparison to control group of the fish.

POLLUTION INDUCE PEROXIDASE ACTIVITY IN GILLS, MUSCLE, HEPATIC AND RENAL TISSUES OF RIVERINE *LABEO ROHITA*

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Aquatic pollution due to heavy metal contamination is the cause of oxidative stress in aquatic organisms that leads to death of an organism. For protection from the oxidative stress every aerobic organism possesses antioxidant defense system. Peroxidase is an important part of antioxidant defensive system that protects an organism from oxidative stress by damaging reactive oxygen species (ROS). To measure the level of peroxidase in relation to various selected heavy metals including Zn, Cr, Ni, Co and Cu in gills, liver, kidney and muscle tissues of *Labeo rohita* captured from River Indus was the purpose of this study. After fish sample collection, the selected studied organs were extracted out after deep ventral side cut at the sampling site. The extracted organs were preserved in crushed dry ice box after keeping them in tagged polythene bags for

transportation in laboratory for further analysis. Water samples were also collected from the sampling sites for the studying various physio-chemical parameters and for the detection of selected metal level. In the laboratory the extracted organs were divided into two parts. One part was for enzyme assay at 470 nm and one for metal detection. The enzyme assay organ parts were homogenized in phosphate buffer (pH 6.5). While, the second part of extracted organs were digested on hot plate to determine the level of selected metals by atomic absorption spectrophotometer. Similarly the proposed fish and water samples were also collected from pond ecosystem for comparison. The inferences of the present study should higher peroxidase activity in all selected organs of *L. rohita* as compared to pond fish. The order of peroxidase activity in various organs was observed as liver>kidney>gills>muscle tissues in riverine *L. rohita* while liver>kidney>muscle tissues>gills in cultured *L. rohita*. Significant difference was observed among different sources fish peroxidase activity statistically at $p \leq 0.05$. The order of selected metals was observed as Cr>Zn>Cu>Ni>Co, Zn>Cu>Cr>Ni>Co, Zn>Cu>Ni>Cr>Co and Zn>Cu>Cr>Ni>Co in riverine *L. rohita* gills, liver, kidney and muscle tissues, respectively while Zn>Cu>Cr in cultured *L. rohita* gills, liver, kidney and muscle tissues, respectively. The inferences of the present study will be helpful in understanding how oxidative stress biomarkers can be used in metal contamination detection.

EFFECT OF Pb + Cr METAL MIXTURE TOXICITY ON SERUM BIOCHEMISTRY OF *LABEO ROHITA*

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Over the last few decades, the contamination of aquatic ecosystem with a wide range of pollutants released from domestic, industrial and other man-made activities has become a matter of concern. As a result of this contamination, aquatic life is affected. The purpose of the present study was to ascertain the cause of Pb+Cr metal mixture on serum biochemistry in *Labeo rohita*. For this purpose, fish fingerlings were purchased from fish seed hatchery, Faisalabad. For acclimatization, fingerlings were kept in cemented tanks. After acclimatization, experimental trial was conducted by keeping fish fingerlings in Pb+Cr metal mixture stressed condition. For comparison, controlled fish were also kept in metal stressed free aquaria. Duration of experimental trial was remained for two weeks. After completing the experimental trial, blood samples were deducting from experimental and control groups. Various hematological parameters were determined from the fish blood samples. Blood was centrifuged at 3000 rpm for 5-8 mints for the separation of serum which was used for the determination of serum biochemical parameters. There was a considerable decline in all serum parameters (protein, Albumin, TC, triglyceride, AST, ALT, ALP, Na⁺, Ca⁺ K⁺ Cl⁻) in the fish blood that was exposed to metal mixture. Statistical analysis showed significant ($p < 0.05$) difference among control and metal mixture stressed *L. rohita* blood serum biochemical parameters.

ASSESSMENT OF HEAVY METAL ACCUMULATION IN MACROINVERTEBRATE SPECIES SAMPLED FROM RIVER RAVI USING ATOMIC ABSORPTION SPECTROPHOTOMETER

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The concentrations of heavy metals (Cu, Mn, Ni) in macroinvertebrates were studied in the water samples and macroinvertebrates sampled from three study sites (Ravi, Confluence of Ravi and QB link canal and QB Link Canal.) using Atomic absorption spectrophotometer. The results showed that the confluence of the two waters showed the best quality of water with low heavy metal concentration, on the other hand, contamination of heavy metals in Ravi was found to be high as compared with Confluence or q-b link canal, due to the polluted runoff from nearby factories like Textile industry and thread industry. A total of 717 specimens of macroinvertebrates were analyzed. Among all the macroinvertebrates sampled from the study sites, the highest concentration of heavy metals were observed of Manganese(0.98ppm) in back swimmers sampled from river Chenab at Balloki followed by Copper (0.71) in orb snail and Nickel (0.668) in backswimmers sampled from river Ravi at Balloki in April 2016. Pygmy back swimmers are a highly tolerant species which showed highest heavy metal concentration and can be considered useful in the cleaning up of effluents before being discharged into the water bodies.

PHYSIOLOGIC STATUS OF REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYMES IN TISSUES OF DIFFERENT FISH SPECIES FOLLOWING EXPOSURE TO FIPRONIL PESTICIDE

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Fipronil, a widely used pesticide that is used for veterinary, residential, and agricultural pests, blocks GABA receptors in the central nervous system. Fipronil is highly toxic to aquatic organisms like invertebrates, fishes and also fish eating birds. It affects physiologic status of proteins and biochemical parameters. Two fish species, common carp and rohu were divided into six groups, each containing ten fishes. They were exposed to sub-lethal concentrations of fipronil (38% i.e 243 µg/L and 65% i.e 415 µg/L of LC₅₀ 639 µg/L) as low and high dose respectively for common carp and (38% i.e 202 µg/L and 65% i.e 345 µg/L of LC₅₀ 531 µg/L as low and high dose respectively for rohu along control group for each species. After 96 h of treatment, tissues including brain, gills, liver, kidneys, muscle and skin were dissected out for biochemical examination of oxidative stress that included protein quantification, estimation of ROS and TBARS, and activities of antioxidant enzymes including the SOD, CAT, POD and GSH. One-way ANOVA and t-test were applied to compare results. P<0.05 was considered significant difference. Results showed severe alterations in tissues of both fish species. In common carp, total proteins showed significant decrease whereas, ROS levels were significantly increased in liver and muscle at low and high doses respectively. While in rohu, a significant increase was found in the ROS levels of brain and skin tissues at high dose and in liver at both doses. Significant increase was

observed in TBARS in brain at both doses, in gills and liver at low dose and in skin at high dose, while highly significant increase was observed in the kidney tissue of common carp. In rohu, level of TBARS increased significantly in brain, gills, liver and skin at both doses, in kidneys and muscles at high dose. In common carp, SOD increased significantly in brain, liver, kidney and skin at low dose. In rohu, SOD decreased in brain, liver, kidneys at high dose and increased in skin, muscles and gills at both doses. In rohu, CAT increased significantly in gills and skin while decreased in liver and kidney tissues. POD increased significantly in brain, kidneys, liver and skin at low dose in common carp while in rohu, significant decrease occurred in brain at low and high doses, in kidney and liver at high dose and increased in gills, muscles, and skin at low dose. In common carp, GSH decreased significantly in muscles, at high dose and in skin at low dose while in rohu, significant increase was observed in gills and in muscles, at high dose. Our findings indicate that rohu is relatively more sensitive to toxic exposure than common carp. It is concluded that fipronil even at low concentration adversely affects the physiologic status of antioxidant enzymes and enhances mortality. The results obtained reveal that fipronil may affect the wild population of fish by inducing oxidative stress and modulating stress response.

X- RAY INDUCED GENOTOXICITY IN MULTIPLE ORGANS OF ALBINO MICE

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Energy Travels from one place to another through radiations. Ionizing radiations are those that can ionize upon striking an atom. Ionization results in removal of electron from a specific atom. X- Rays and Gamma Rays are two major types of Ionizing radiations. During the last few decades the use of X- Rays, particularly in health, has been increased at alarming rate. These are used for diagnostic and treatment of various diseases. Different studies have been conducted to explore wide range of impacts of these radiations on different organisms. However little information is available about the genotoxicity of these rays particularly with reference to the developing and under developed countries. Keeping in view study was designed to explore the impacts of these radiation on the different organs of the mice. Mice were randomly divided into four groups and named as X1, X2, X3 and C. Each group contains 10 animals. X1, X2 and X3 were exposed to 60 kV, 70kV and 80 kV of X-Rays respectively. Fourth group was not exposed with any radiations and it served as control. After exposure to x-rays blood was isolated. Mice were dissected, organs (liver and lungs) were minced and protein removal was done by single cell suspension. Results of comet assay indicated that maximum DNA damage values were obtained with 80 kV of X-Rays. It means DNA damage depends upon dose of ionizing radiation (X-Rays). The results of the present study will be helpful to indicate the DNA damage caused by X- rays in liver, lungs and lymphocytes of albino mice.

GENOTOXICITY OF GAMMA RAYS IN LIVER, LUNGS AND LYMPHOCYTES OF ALBINO MICEZARINA IQBAL,¹ TAYYABA ALI,¹ MUHAMMAD ISMAIL² AND FARKHANDA ASAD¹¹*Department of Zoology, Government College University, Faisalabad, Pakistan.*²*Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan.*

Radiation is a process that allows movement of energy from one place to other. In the last many years use of different types of ionizing radiations in diagnosis and treatment of various diseases has been increased. These radiations contain enormous amount of energy. When they strike on any living organism they penetrate into that organism crossing cell membrane and ultimately they reach the DNA causing damage to the DNA. Contact to ionizing radiation can cause damage to living tissues, which can result in mutation and some mutations may lead to cancer. A study was designed to detect and compare the impact of Gamma Rays on the liver, lungs and peripheral lymphocytes of the albino mice. Single cell gel electrophoresis assay commonly known as comet assay was used to detect the damage to the DNA. Albino mice were used in the study and were randomly divided into four groups and named as G1, G2, G3 and C. Each group contains 10 animals. G1, G2 and G3 were exposed to 1cGy, 3cGy and 5cGy of Gamma rays respectively for 1min. Group C was not exposed with any radiations and it served as negative control group. Immediately after exposure to gamma rays peripheral blood was isolated, mice were dissected, organs (liver and lungs) were minced and were subjected to comet assay. Significant damage ($P < 0.001$) was observed in all three doses in liver, lungs and lymphocytes when compared with control. G1 and G2 were not significantly different from each other, however there was significantly higher DNA damage in G3 as compared to G1 and G2. Results of the present study indicate that even low doses of Gamma rays for short period are capable of causing DNA damage in blood and different organs of the mice. Damage further increase with increase in intensity.

EFFICIENCY OF ARTEMISIA VULGARIS AND BERGINIA CILLIATA EXTRACT AGAINST DIETHYL-NITROSAMINE (DEN) INDUCED TESTICULAR CHANGES IN MICES. SHAMIM,¹ S. JABEEN,¹ T. AZIZ,² H. U. WAZIR,² M. Z. ANJUM¹ AND S. AKHTER^{1*}¹*PMAS-Arid Agriculture University, Murree road, Post code 46000, Rawalpindi.*²*Quaid-e-Azam University, Islamabad *shamim@uau.edu.pk*

Diethylnitrosamine (DEN) or N-Nitrosodiethylamine (NDEA) is being used as a carcinogen in experimental animal model systems. The DEN treatment at a necrogenic dose can cause acute toxicity to various cell types in the adenohypophysis of the pituitary gland and alter serum levels of several hormones that can affect reproduction. *Berginia cillliata* and *Artemisia vulgaris* are known for their antioxidative and protective effects against a variety of chemicals. The present study aims at elucidating the anti-carcinogenic activity of *Artemisia vulgaris* and *Berginia cillliata* against diethylnitrosamine (DEN) induced changes in testes of albino mice. Four to five weeks old experimental mice (N=120) having average weight 35-40g were kept under standard uniform conditions. Mice were randomly divided into six groups (20 mice/group) and data on initial body weight was recorded. Mice were treated once in a week with saline (control) (3.5 µl/g), DEN alone (3.5 µl/g), *Artemisia vulgaris* extract alone (150 mg/kg), *Berginia cillliata* extract

alone (150 mg/kg), DEN+*Artemisia vulgaris* extract (mice were treated with DEN first eight weeks then treated with *Artemisia vulgaris* next eight weeks) and DEN+*Berginia ciliata* extract (mice were treated with DEN first eight weeks then treated with *Berginia ciliata* next eight weeks) with same quantity. Doses were given for sixteen weeks. At the end of experiment, the final body weight was recorded, the blood samples were collected and mice were dissected for study of testicular morphology and histology. Testes were fixed in buffered formaline for one week then dehydrated and stained in eosine and heamatoxylin and mounted with DPX. Histological images were analyzed by image J software. Significant reduction ($P < 0.05$) in body weight was observed in mice treated with DEN and *Artemisia* co-treated group while body weight was not affected in *Berginia* co-treated group. Similarily plasma testosterone was observed significantly reduced ($P < 0.001$) in DEN alone treated group and DEN + *Artemisia* treated groups compared to control, *Artemisia vulgaris* extract alone, *Berginia ciliata* extract alone, and DEN+*Berginia ciliata* extract respectively. Histological evaluation revealed significant reduction in diameter of seminiferous tubules, epithelial height and cell numbers in DEN treated group compared to the control. These effects were restored in the group treated with *Berginia ciliata* extract but were not restored in *Artemisia vulgaris* extract co-treated group. In conclusion, results of the present study revealed that *Berginia ciliata* extract is more potent than *Artemisia vulgaris* extract in reversing DEN induced reproductive toxicity in male mice. Further studies are needed to characterize the specific components of *Berginia ciliata* that are useful in treating reproductive organ toxicity.

OXIDATIVE STRESS BIOMARKERS IN ASSESSING CADMIUM AND LEAD TOXICITY IN THE *CHANNA MARULIUS* AND *WALLAGO ATTU*

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Heavy metals and their salts are considered as very important group of environmental toxicant which in small quantities may be essential nutrients that protect our health, yet in larger quantity it being toxic and hazardous to all living organisms including aquatic organisms. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. Heavy metals are important inducers of oxidative stress in aquatic animals, promoting formation of reactive oxygen species (ROS). The present study was planned to assess the metals induced oxidative stress in *Channa marulius* and *Wallago attu*. After the determination of LC₅₀ 96-hr and lethal values of cadmium and lead to *Channa marulius* and *Wallago attu*, the fish organs like liver, kidney and gills were used to estimate the antioxidant enzyme assay (Catalase) from the metal treated fishes. Catalase activity decreased with increasing metallic ion (Cd, Pb) concentrations in the test mediums for both fish species that indicate stress conditions in fish. Maximum activity of catalase was noted in control (without metal exposure) fish than metal exposed fish. The result demonstrates that alteration in the antioxidant enzymes reflects the presence of heavy metals which may cause oxidative stress in *Channa marulius* and *Wallago attu*. The study therefore, provides a rational use of biomarkers of oxidative stress in biomonitoring of aquatic pollution.

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 H₂O₂ into H₂O and O₂ molecule at 240nm with the help of spectrophotometer. The inferences of the present study revealed higher hepatic (134.3±0.305 U mL⁻¹) and renal (121.07±1.37 U mL⁻¹) catalase enzyme activity in pond fish compared to the riverine fish liver (107.2±0.435 U mL⁻¹) and kidney (104.4±0.41 U mL⁻¹) 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 inferences it is concluded that biomarkers serves as an indicator of metal contamination in aquatic environment.

IMPACT OF HEAVY METAL (CADMIUM) TOXICITY ON MORPHOLOGY AND HISTOPATHOLOGY OF *MILLARDIA MELTADA*

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Study was carried out on field rats (*Millardia meltada* spp.) collected from fields around Faisalabad. On the basis of low and high amount of cadmium concentrations, rats were divided into two equal groups. Group1 and 2 were served with 15mg/kg and 30mg/kg body weight/day respectively in feed and drinking water for two months. Group 3 was kept as control. After every

week of treatment rats were dissected. In cages, acclimatization of rats was done for a period of one week before starting the experiment. Morphological changes, food and water intake and weight were checked on daily basis. Commercial diet was given to rats during this experiment. From the rats of each group after every one week period tissues samples were taken. Analysis of different tissues was done to evaluate the treatment responses. Rats given low cadmium showed only slow response to stimulus and little change in fur texture. Due to high amount of cadmium, rats became sluggish while fur color and texture also changed. Feed and water intake in cadmium treated rats also decreased. Histopathological changes were observed in treated rats. Liver and kidney cells structure and function was not normal as compared to control group. Hemorrhagic spots, widespread fibrosis, interstitial mononuclear cellular infiltration were observed in liver of treated rats. In the renal tissues, Necrosis, dilation, calcinosis occurred in tubules, and massive local haemorrhage and destruction of the basement membrane occurred in treated group. Reduction occurred in the Hb, RBC, Hct, WBC's and MCH parameters while increase in the values of MCV and MCHC parameters. Mean Cadmium accumulation in liver and kidney of treated rats increased with respect to exposure period and way of exposure.

STUDIES ON THE GENOTOXICITY OF ALUMINUM TO *LABEO ROHITA*

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Metals are the major pollutants in the aquatic environment. A lot of research literature is available globally about the accumulation of heavy metals in the aquatic ecosystems as well as in different body organs of aquatic animals especially fish. Heavy metals mainly produce oxidative stress in aquatic animals that lead to production of reactive oxygen species. These ROS cause oxidative damage to the different tissues and cellular components like protein and DNA. Present study was conducted to determine the toxic effects of aluminum on DNA damage in terms of %age damage, genetic damage effect and cumulative tail length of comets and micronuclei induction in fish peripheral blood erythrocytes. For this purpose, *Labeo rohita* was exposed to four sublethal concentrations (17%, 25%, 33% and 50% of 96-hr LC₅₀ of aluminum) of aluminum for a period of 30 days in controlled laboratory conditions. Results revealed significant toxic effect of this metal on fish genetic material of fish. Among four sublethal concentrations, 50% of 96-hr LC₅₀ of aluminum caused significantly higher %age damage, genetic damage effect and cumulative tail length of comets with the mean values of 66.67±4.16 %, 1.93±0.14 and 168.09±0.46 µm, respectively. However, frequency of micronuclei was significantly higher due 50% of 96-hr LC₅₀ exposure of aluminum which was statistically (p<0.05) similar to micronuclei frequency caused by 33% of 96-hr LC₅₀ of aluminum. The findings of this experiment showed that aluminum caused carcinogenic effects on fish. Moreover, Comet assay and micronucleus test appeared reliable indicator of metallic ion pollution in aquatic environment.

EMBRYOTOXIC EFFECTS OF CIPROFLOXACIN IN CHICKEN

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Ciprofloxacin is considered as a relatively safe drug having broad spectrum antimicrobial activity in humans and animals, besides its reported cartilage damage in children and debated use in pregnancy in humans. Current study is designed to highlight the embryotoxic effect of ciprofloxacin in chicken. Fertilized eggs (from healthy chicken, grown under standard conditions) were taken, grouped in four categories having 8 eggs each. On 4th day of incubation, group 1 and 2 were injected with Ciprofloxacin (30 and 60 mg per egg respectively). Group 3 was injected with needle only and group 4 was injected with sterilized saline solution. Group 5 was kept as a control, without any injection. On the 18th day of incubation, the growth of embryos was monitored. Body weight of embryo, formation of beak, eyes, palate, skull, feathers, limbs, claws, nails, and size of head were analyzed and compared in both treated and untreated group. Results were statistically analyzed by chi square test. Hemorrhages on neck and head areas were noticed in treated group with high doses of ciprofloxacin (60mg/egg). Low body weight along with the defective beak and nail formation was also noticed. No other clear external deformity was observed in any treated and normal groups. Hence in the light of obtained results, embryotoxic effect of ciprofloxacin can not be ignored. It can be inferred that high or repeated doses of this drug can reduce the turn over number of successful hatching chicks. The consumption of antibiotics treated chicken, can lead to the development of antimicrobial resistance in humans. A comprehensive toxicological study is required to analyze the cardiovascular defects, spina bifida, polydactyly, and hypospadias in farm animals.

DETECTION OF PCR MARKER IN PAKISTANI CANNABIS COULD BE ABLE TO RESTRICT DRUG TRAFFICKING FROM AFGHANISTANISHRAT AZIZ,^{1,*} MEHREEN MUFTI,¹ MEHWISH KHAN² AND SAADAT ALI³¹*University of the Lahore*²*University of the Punjab*³*University of Veterinary and Animal Sciences, Lahore, Pakistan*

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The big road of drug trafficking from Afghanistan to remaining world passes from Pakistan. From forensic point of view, it is very important for Pakistan to have a clear position in this matter. Hence, accurate identification of drug material in seized samples is crucial. In the present study, the gene for THCA synthase coding region was amplified by PCR and the products were sequenced to design a PCR marker for verifying differences between Pakistani "fiber type" and "drug type" cannabis varieties and their alterations from the western world. The coding region of THCA synthase gene was amplified by PCR. Sequence alignment of our representative stocks (C, M, L1, L2, L3) was done and compared with reference sequences of published fiber and drug types. The nucleotide sequences of these representative stocks were successfully determined (European Nucleotide Archive database accession numbers: LN998179, LN998180, LN998181, LN998182 and LN998183). Novel differences have been observed which are used for suggestion of PCR markers specific for Pakistani cannabis.

**EFFECT OF SOME PYRETHROIDS AND ORGANOPHOSPHATES AND THEIR
IMPACT ON THE ACTIVITY OF ESTERASES AND PHOSPHATASES IN HOUSE FLY
(*MUSCA DOMESTICA* LINN.)**

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The house fly, *Musca domestica* L., is an important ectoparasite with the ability to develop resistance to insecticides used for their control. Now-a-days, insecticide resistance is a major problem in the management of public health pests. The aim of the current study is to evaluate the resistance of currently used insecticides such as pyrethroids, deltamethrin (1.5% EC), alpha cypermethrin (10% SC), permethrin (0.5% WP) and organophosphate (DDVP 50% EC) in *Musca domestica*. Insecticide bioassays carried out by exposing the larval stage of *Musca domestica* to different insecticide doses through impregnated filter paper and a residual film in petri plates. Mortality data recorded after 24, 48 and 72 hours of treatment. Resistant insects evaluated on the basis of their LC₅₀ values. The activity of esterases and phosphatases evaluated in order to assess the resistance in house fly. Both the enzymes were found inhibited compared to control. Biotic potential (length of developmental period) was measured for susceptible (S) strain against different concentrations of deltamethrin (2.5, 5, 10, 20, 40) µg/ul. The R strains showed an 11 to 43% decrease in biotic potential. In contrast, the biotic potential of control group was seldom different from the S strain. An increase in pesticide concentration caused delay in developmental period. The current study revealed the resistance status of organophosphates and pyrethroids against *Musca domestica* which would help facilitate to carefully use these insecticides for its management.

**ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES THROUGH
ECONOMICAL METHODS AND ASSESSMENT OF TOXICITY THROUGH
OXIDATIVE STRESS ANALYSIS IN *LABEO ROHITA***

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The physicochemical and biological properties of metals changes as the particles are reduced to nanoscale. This ability increases the application of nanoparticles in commercial and medical industry. Keeping in view this importance, the Ag-NPs were synthesized by reduction methods using the formaldehyde as reducing agent in the chemical route and lemon extracts in the biological route. The SEM images of nanoparticles suggested that the particles were agglomerates, spherical in shape with mean diameter of 16.59 nm in chemical route and 42.93 nm in the biological route. The particles were between the ranges of 5-80 nm with maximum frequency between 5 to 20 nm in chemical route and between the ranges of 5-100 nm with maximum frequency between 15 to 50 nm in biological method. In the second phase of the study, the effect of Ag-NPs on the oxidative stress was studied. For this purpose, *Labeo rohita* (20±2.5 g weight and 12±1.4 cm in length) were involved. Six treatments were maintained in three replicates having 5 fishes in each replicate. First treatment was used as control group and other five treatments were

exposed to either 10 or 20 or 30 or 45 or 55 mgL⁻¹ for 28 days. The treatment of Ag-NPs caused oxidative stress in the liver and gill tissues which was led by changing the activities of antioxidant enzymes. The level of CAT was decreased in response to Ag-NPs concentration in dose dependent manner. Ag-NPs treatment stimulated the liver and gill tissues to significantly increase the level of SOD which might be due to synthesis of SOD and addition in the pre-existing SOD level. The level decreases again due to depletion of SOD level. There was a sharp decline in the activities of GST in both gills and liver tissues even at lower concentration and this decrease in the GST activity was significantly different at each treatment after 28 days of treatment except 20 mgL⁻¹. The MDA levels of gills and liver tissues were increased with the increase in the concentration. The elevated levels of GSH showed that the liver started defensive mechanism against the oxyradicals. This study finds out the cheap eco-friendly and economical method of Ag-NPs synthesis. It is further revealed that Ag-NPs caused oxidative stress in the aquatic animals if exposure occurs at high concentrations.

SUBLETHAL TOXIC EFFECTS OF MERCURY ON PROTEIN OF *LABEO ROHITA*

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Present study was planned to investigate LC₅₀ at 96 hours and protein estimation in liver and muscles of *Labeo rohita*, treated with mercuric chloride respectively with 0.1, 0.25, 0.50, 1.0mgL⁻¹ concentrations in three replica and one as a control. The LC₅₀ was 0.36±0.04 mgL⁻¹. In the chronic phase fish treated with mercuric chloride respectively with 0.05, 0.10, 0.15, 0.20 mgL⁻¹ in three replica for 14 days. There was significant decrease in protein contents of liver and muscles of the affected fish as compared to the control. The need of the day is to have research on fish health, diseases and protection so that fish industry can grow economically in Pakistan.

COMPARISON OF HEAVY METAL CONCENTRATION IN WATER AND IN SOME SELECTED TISSUES OF THREE FISH SPECIES *CATLA CATLA*, *LABEO ROHITA* AND *CIRRHINUS MIRIGALA* PURCHASED FROM LOCAL FISH MARKET

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Fish is important source of proteins, minerals, vitamins and unsaturated fatty acids. With increase in population the water pollution is also increasing due to human activities. The water pollution is mainly due to heavy metal. These heavy metals cause serious health problem. Heavy metals are present in aquatic environment and assembled in fish tissue. From fish these enters in the food chain and incidentally it is a risk for people who used this effected fish. Ingestion of heavy metals through food and its accumulation in organisms, causes serious health problems such as injury to the kidney, symptoms of chronic toxicity, renal failure and liver damage and also damage to bones, the brain, blood, and the thyroid glands. The concentration of heavy metal (Cd, Cr, Cu, Zn, and Pb) was measured in gills, liver, muscle and intestine of three different species

of fish (*Catla catla*, *labeo rohita* and *Cirrhinus mirigala*) purchased from the local fish market. The water sample from the same ponds is also collected and analyze for the metal concentration. The concentration was measured by the use of atomic absorption spectrophotometer by wet digestion of sample. Research indicates that the level of Cd was lowest in all of three fish species organ like muscle, liver, intestine and also gills as well as in water. Muscle of *catla catla* show highest concentration of Cr, 3.57 mg/kg as compared to other tissue of three fish species. *Labeo rohita* show highest concentration of Cu, 21.90 mg/kg than any other fish. Lowest concentration of all these metal was found in *cirrhinus mirigala*. One way ANOVA is use to compare the metal between the different species and their organs (significant values, $p < 0.05$).

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

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The growth performance, condition factor, feed intake and feed conversion efficiency of 240-day three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* under chronic (1/3 of LC₅₀) exposures of Aluminium (Al) were determined. The fish were fed with feed (34% DP and 3.00 Kcalg⁻¹ DE) to satiation daily. At termination of each trial, the fish were dissected and their organs viz. bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats isolated for the determination of Al concentrations. During growth trials under Al sublethal stress, *Catla catla* gained significantly higher weight of 19.60 ± 0.45 g, followed by that of *Cirrhina mrigala* (18.81±0.55 g) and *Labeo rohita* (18.10 ± 0.48 g). All the three control (un-stressed) fish species exhibited significantly better growth due to significantly higher feed intakes than those grown under sub-lethal concentrations of Al. The sublethal exposure of Al to the fish caused significantly variable accumulation of metal in the body organs of three fish species. Fish liver, kidney and gills accumulated significantly higher Al as 47.54 ± 3.14, 44.99 ± 3.11 and 35.23 ± 1.17 µgg⁻¹, respectively than the other organs of fish.

DETERMINATION OF HEAVY METALS IN SOME COMMON FISHES SPECIES OF KARACHI HARBOUR (WEST WHARF) WHITE POMFRET (*PAMPUS ARGENTEUS*) AND RED SNAPPERS (*LUTIJANUS CAMPECHANUS*)

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The aim of this research is to measure the concentration level of heavy metals like Lead (Pb), Cadmium (Cd) and Chromium (Cr) in two marine fishes red snappers (*Lutjanus campechanus*) and white pomfret (*Pampus argenteus*), were collected from two sites of Baluchistan (Ormara and Pasni), which are transported to Karachi Fish Harbor for selling. The metals like Cadmium (Cd), Lead (Pb) and Chromium (Cr) were found in very little amount in both fishes and all these three metals detected are less than the standard limit of WHO. Fish samples were collected from the given selected sites. They were brought to laboratory in polythene bags. The process of

digestion weighed dried sample material (muscles portion) of 5gm was then transferred to china dish and digested by the addition of concentrated 5 ml Nitric acid and 5 ml concentrated perchloric acid. Metal estimation in the digestions was carried out using Atomic Absorption Spectrometer (ASS) 3100 of Perkin Elmer. The selected fishes of both location (Ormarr and Pasani) of Baluchistan shows less pollution in these areas, and the selected fishes are safe for human consumption.

BIOLOGICAL EVALUATION OF SPORTS DRINK USING SPRAGUE DAWLEY RATS

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The project was designed to develop whey based fermented sports drink followed by bio efficacy studies. The physico-chemical analyses of sports drink determined high pH (4.35 ± 0.35), crude protein (0.87 ± 0.04 %), fat (0.20 ± 0.01 %), lactose (4.11 ± 0.31 %) and total solids (14.76 ± 0.81 %) while total plate count (TPC) was slightly high ($5.74 \pm 0.25 \times 10^4$ cfu/mL) in whey based sports drink. The results regarding mineral analysis indicated that whey contain appreciable amount of calcium, magnesium, sodium and potassium. Furthermore, amino acid profile elucidated it a complete and balanced source of essential amino acids. Amongst essential amino acids, highest values was recorded for branched chain amino acids like leucine (72.48 ± 4.79) followed by lysine (59.17 ± 2.68) and valine (43.72 ± 1.52) mg/g protein. Drink having Cheddar whey with additional fermentation was further selected on the basis of nutritional and physico-chemical analyses for biological evaluation. Biological evaluation of selected sportsman drink revealed highest total digestibility (96.71 ± 1.65 %), biological value (98.01 ± 1.55 %), net protein utilization (87.31 ± 0.86 %), protein efficiency ratio (3.42 ± 0.09), net protein ratio (5.91 ± 0.08 %) and protein digestibility corrected amino acid score (97.57 ± 2.01 %).

EFFICACY OF PLANT EXTRACTS AND MICROBIAL BIOPESTICIDE TO EVALUATE GENOTOXICITY IN *DROSOPHILA MELANOGASTER*

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The fruit fly, *Drosophila melanogaster* is a versatile model organism extensively used in the field of genetics and developmental biology. Recently, *Drosophila* has been described as a pest species of homes, resultants and fruit markets. Keeping in view; the current study was designed to estimate the effectiveness of different weed plant extracts in combination with microbes against *Drosophila melanogaster* under laboratory conditions. The DNA damage was also evaluated in order to assess the genotoxicity due to plant extract. *D. melanogaster* was reared at room

temperature (25-30°C) using both natural and artificial diet. Five different weed extracts, bacteria (*Bti*), *Pseudomonas aerogenosa* singly and/or in combination with weed extracts were used during the study. Percentage mortalities of *D. melanogaster* were calculated, the data of mean corrected mortality, repellency was subjected to ANOVA using statistica 13.0 for windows and LC50 of all the tested biopesticides were determined using Probit Analysis Program (version 1.5). The most significant biopesticides out of five weed plants extraction protocol. DNA concentrations were measured by using spectrophotometer. Random primers were selected and amplified accordingly using Polymerase Chain Reaction (PCR). The PCR products were considered using agarose gel electrophoresis. The genetic data was analysed using POPGENE software. Comet assay was also performed for genotoxicity according to given protocols. Based on the results, an environment friendly, feasible and more economical biopesticides could be introduced to devise an integrated control of fruit flies.

MOLECULAR BASIS OF Cd⁺² STRESS RESPONSE IN *CANDIDA TROPICALIS* 3Aer

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Current study is elucidating the bioremediation potential and cadmium-induced cellular response with its molecular basis in *Candida tropicalis* 3Aer. Spectroscopic analysis clearly illustrated the involvement of yeast cell wall components in biosorption whereas bioaccumulation was confirmed by TEM, SEM and EDX scrutiny. TEM images divulged extracellular as well as cytoplasmic and vacuolar cadmium nanoparticle formation, further validated by presence of *ycf1* gene and increased biosynthesis of GSH under cadmium stress. Transcriptomic and proteomic approaches have rarely been applied to study change in cell architecture under environmental stress conditions, but this study is unveiling the altered expression of proteins and genes in *C. tropicalis* 3Aer under cadmium stress in concentration and time dependent manner, respectively. Fourteen proteins exhibited differential expression and found involve in cellular redox homeostasis, nitrogen metabolism, nucleotide biosynthesis and carbohydrate catabolism. Interestingly, *C. tropicalis* 3Aer is additionally equipped with nitrile hydratase enzyme, rarely been reported in yeast and thus have potential to remove nitriles (extremely toxic compounds) from environment. Cd⁺² toxicity not only caused growth stasis but also upregulated the cysteine biosynthesis, protein folding and cytoplasmic detoxification response elements. Moreover, prolonged Cd⁺² cytotoxicity resulted in chromatin remodeling and most probably the epigenetic alterations.

STUDY ON AMELIORATIVE EFFECTS OF SODIUM SELENITE IN CADMIUM CHLORIDE INDUCED RENAL TOXICITY IN MALE SPRAGUE DAWLEY RATS

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This study explored the nephrotoxic effect of cadmium on the kidney of male Sprague Dawley Rats and ameliorating effect of Selenium. Cadmium is an environmental hazard for humans and animals and is a relatively rare, soft, bluish-white, transition metal that occurs with zinc ores. Cadmium causes the carcinogenic effects on kidney, liver, testis, brain and different body organs. Selenium is a critical constituent for antioxidant activities and play vital role in biological

process of humans and animals. The study assessed the effect of Cadmium chloride or sodium selenite alone or in combination on the kidney of male albino rats. Male Sprague Dawley rats were distributed into four groups, exposed to either normal saline solution (control group) or the sub-lethal dose of cadmium chloride or sodium selenite alone or in combination at the dose of 1mg/Kg body weight of rat for 28 days. Animals were weighed and sacrificed after 28 days of exposure. Control group showed the normal histology with normal glomerulus, epithelial lining, renal tubules and healthy nucleus. Cadmium Chloride treated group showed dilation in renal tubules, severe necrosis, vacuolization and distortion of epithelial lining and nucleus degeneration. Selenium treated group showed the normal structure in renal tubules, reduced vacuolization and apoptosis. Cadmium chloride and sodium selenite in combination showed the vacuolization, apoptosis, necrosis, and constricted glomeruli. Metal concentration analysis showed high levels of Cadmium in Cadmium Chloride treated group and more Selenium was found in Sodium selenite exposed rats. We concluded that cadmium exposure results in severe damage, carcinogenesis, necrosis, nucleus distortion and dilation in glomerulus in the kidney tissues. Selenium treatment was able to shows some tissues amelioration at the rate of 1mg/kg b.w.

8. HERBAL MEDICINE

MEDICINAL VALUE OF *TRIANTHEMA PORTULACASTRUM* (AN *EX SITU* STUDY ON RABBITS)

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Objective: To study medicinal value of whole plant of *Trianthema portulacastrum*. **Materials and methods:** Medicinal value of *T. portulacastrum* was evaluated using CCl₄ as liver toxicant in rabbits. The potency of Ethanolic and Aqueous extracts of plant was compared with that of silymarin at a dose of 75mg/kg, 125mg/kg and 225mg/kg, P.O. The SGOT, SGPT, TC, TG, LDL-C, and HDL-C and bilirubin levels of blood were measured spectrophotometrically. **Results:** Ethanolic extract of whole plant caused significant fall in the enzyme levels in serum in rabbits. **Conclusion:** *T. portulacastrum* has shown significant activity in rabbits toxicated with CCl₄ and can be recommended for further studies.

IN VITRO SCREENING OF ANTIBACTERIAL AND DNA DAMAGING EFFECT OF *PERSICARIA AMPLEXICAULIS* EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

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In current research antibacterial effect of *P. amplexicaulis* rhizome extracts was analyzed against *Escherichia coli*, *Serratia marcescens*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* through agar well diffusion method. Phytochemical screening, antioxidant potential, thin layer chromatography, bioautography, and spot screening were also analyzed. Genomic DNA was extracted from bacterial strains to check the effect of *P. amplexicaulis* on DNA synthesis inhibition. Antibacterial results through agar well diffusion revealed that DMSO extract indicated significant inhibition of *S. marcescens* (11.33±0.57 mm), ethanol and acetone extracts indicated moderate sensitivity (both 6.66±0.57 mm), diethyl ether extract gave low sensitivity (1.0±0 mm) while the extract of methanol showed no sensitivity. For *S. pyogenes*, all rhizome extracts showed no sensitivity. For *P. aeruginosa*, only DMSO extract of rhizome indicated highest sensitivity

(10.0±1.0 mm), methanolic extract showed low sensitivity (1.0±0.0 mm). Through genomic DNA analysis it was recorded that methanol and ethanol extracts reduced the quality of genomic DNA compared to control (without treatment and solvent treated). In all extracts no DNA fragmentation was observed. On the other hand, growth characteristics such as turbidity, sedimentation/precipitation and flocculence of bacterial pathogen were also observed when both control solvents and extracts were applied. Various phytochemical constituents such as ketoses, oligosaccharides, amino acids, amines, sugars, phenols, flavonoids, and antioxidant constituents were detected using ninhydrin test, urea test, AlCl₃ spray, and p-anisaldehyde spray. Direct bioautography against *P. aeruginosa*, *K. pneumoniae*, *S. pyogenes* and *S. aureus* using dipping suspension and agar-overlay methods showed positive results. It was observed that ethanol, methanol and diethyl ether rhizome extracts showed stronger ABTS⁺ scavenging potential, with potential values of 91.9 %, 83.4 % and 83.7 % respectively. It was concluded, that the extracts of *P. amplexicaulis* can be effectively used as potential antimicrobial and antioxidant agents to overcome the problem of bacterial infections and multidrug resistant microbial strains.

REPLACEMENT OF ANTIBIOTICS WITH *LINUM USITATISSIMUM* AND *SESAMUM INDICUM* EXTRACT AS GROWTH PROMOTORS IN DIETS FOR FINGRLINGS OF GRASS CARP *CTENOPHARGODON IDELLA*.

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A 90-day feeding trial (09-06-2014 to 09-09-2014) was designed to investigate the effects of dietary supplementation of *Linum usitatissimum* and *Sesamum indicum* seed extracts on hematological traits and growth of *Ctenophargodon idella* fingerlings. Fish were housed in cemented rectangular tanks. There were three treatments and a control group. Control diet contained 32% protein and was composed of conventional ingredients. 02% of the total ingredients were replaced with extract of *Linum usitatissimum* seed extract in treatment 1 and 2% of the total ingredients were replaced with extract of *Sesamum indicum* seed extract in treatment 2 respectively. Where treatment 3 composed of 96% of control diet + 2% *Linum usitatissimum* seed extract + 2% *Sesamum indicum* seed extract. Feed was offered to fish @ 2% of its body weight. Initial and final weight of *Ctenophargodon idella* fingerlings was 7.33g, 15.98gm in control, 7.14 gm, 17.38 gm in T₁, 7.27 gm, 16.49gm in T₂ and 7.30 gm, 16.86 gm in T₃ respectively. All treatments showed better growth than control but treatment composed of linseed oil in diet showed significantly higher growth. Hematological studies showed increased levels of Hemoglobin, Platelets, TLC, RBCs, HCT, WBCs, and MCH in treatments while the level of Lymphocytes & MCV is better in control diet as compared to treatments. Fish were housed in cemented rectangular tanks with stocking density of 126.05mg/liter, meaning 31-33 liters of water per fish. Fish fed diet with 2% linseed extract in feed showed highest weight gain (10.24g) best F.C.R (1.28) and good hematological results suggested that *Linum usitatissimum* seed extract is better option to include in future feed formulations for *Ctenophargodon idella* for maximum performance and minimum feed wastage.

IN VIVO ASSESSMENT OF ANTICARCINOGENIC ACTIVITY OF *BERGENIA CILIATA*

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Bergenia ciliata has potent antioxidant activity, radical scavenging capacity and anticancerous properties. Hepatocellular carcinoma (HCC) is the most frequent primary malignancy of liver, and accounts for as many as one million deaths worldwide in a year. The aim of the present study was to evaluate the anticancerous efficiency of *B. ciliata* rhizome against diethylnitrosoamine (DEN) induced hepatocarcinogenesis in balb c mice. Mice were classified into three groups as follows: First group a control group containing seven mice received saline solution (3.5 µl/mg), second group having fourteen mice received diethylnitrosoamine (3.5 µl/mg) intraperitoneally once in a week for eight consecutive weeks while, third group having seven mice received plant extract (150 mg/kg body weight) once in a week. After eight weeks of DEN induction group II mice were divided into two subgroups containing seven mice each, one subgroup was sacrificed while rest of the subgroup was treated with plant extract (150 mg/kg) once in a week for eight consecutive weeks. The model of DEN injected hepatocellular carcinomic (HCC) rats elicited significant decline in levels of albumin with concomitant significant elevations in tumor markers aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LDH), alpha feto protein (AFP), gamma glutamyl transferase (Y-GT), 5 nucleotidase (5NT), glucose-6-phosphate dehydrogenase (G6PDH) and bilirubin. The intraperitoneal administration of *B. ciliata* as a protective agent, produced significant increases in albumin levels with significant decreases in the levels of tumor markers AFP, ASAT, ALAT, LDH, Y-GT, 5NT, G6PDH and bilirubin.

**EVALUATION OF ANTIDERMATOPHYTIC ACTIVITY OF *EMBLICA OFFICINALIS*,
ACACIA CONCINNA AND *SAPINDUS TRIFOLIATUS* HERBAL EXTRACTS**

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A study was carried out to screen the antidermatophytic effect of phyto-medicines being natural and cheaper source to treat dermaphytoses. Herbal extracts of *Embllica officinalis*, *Acacia concinna* and *Sapindus trifoliatus* were used in antifungal screening. Alcoholic as well as aqueous extracts were prepared and their different dilution were made viz., 1%, 3%, 5%, 10%, 20%, 30%, 40% and 50%. Three fungal strains (*Trichophyton rubrum*, *Trichophyton mentagrophyte* and *Trichophyton verrucosum*) were revived on Sabouraud 2% dextrose agar and viable count was done by adjusting optical density at 0.5 values. The stock solution was prepared by adding 7 days old culture in 5% dimethyl sulfoxide and then stored at 4 °C -10°C for use. Antidermatophytic activity was screened by Disc diffusion method. Aqueous and ethanolic extracts of *Embllica officinalis* and

Acacia concinna showed antifungal activity against all tested strains and depicted increasingly higher antifungal activities at higher concentrations. *Trichophyton rubrum* was found to be least sensitive to ethanolic extract of both herbs. Aqueous and ethanolic extracts of *Sapindus trifoliatus* has shown antifungal activity relatively at higher concentrations. Aqueous and ethanolic extracts of *Acacia concinna* and *Sapindus trifoliatus* proved to be fungistatic while *Emblica officinalis* has showed fungistatic as well as fungicidal effects at their lower and higher concentration respectively. The present outcomes revealed these phyto-sources to be significantly effective against selected fungal strains

EVALUATION OF HYPOGLYCEMIC AND HYPOLIPIDEMIC PROPERTIES OF *MYRICA ESCULENTA*

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Medicinal plants and their derivatives are in use from very early times to control diabetes mellitus. Complications and exact effectiveness of these drugs are still not completely known. Many works were carried out by use of medicinal plants on rats but use of such plants can be better understood by applying them on humans. In present study a placebo group of Diabetic Type 2 patients were chosen to investigate the effect of *Myrica esculenta* on glucose level and lipid profile. Present study reveal that *Myrica esculenta* reduce 22% glucose level, 7.6% triglycerides, 12.9% cholesterol, and 21.5% LDL cholesterol level in diabetic patients. This investigation can be helpful in controlling diabetes mellitus in cheap price and without any side effect.

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC POTENTIAL OF *SWERTIA CHIRATA*

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Medicinal plants are in use from very early times to control diabetes mellitus. *Swertia chirata* is a medicinal plant with anti-diabetic potential and used in therapeutic herbal preparations. In present study grinded powder of *Swertia chirata* was given to Diabetes Type 2 patients in order to know its effect on glucose level and lipid profile. Present study reveal that *Swertia chirata* reduce 14.5% glucose level, 10.5% triglycerides, 8.6% cholesterol, and 14.4% LDL cholesterol level in diabetic patients. This investigation can be helpful in controlling diabetes mellitus in cheap price and without any side effect.

ORAL SUPPLEMENTATION OF *OCIMUM BASILICUM* HAS THE POTENTIAL TO IMPROVES THE LOCOMOTORY, EXPLORATORY, ANXIOLYTIC BEHAVIOR AND LEARNING IN ADULT MALE ALBINO MICE.

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The aim of this project was to determine the effect of 100 mg/ ml solvent/ Kg body weight of *Ocimum basilicum* leaf extract on neuromuscular co-ordination, exploratory, locomotory and short term memory formation in male albino mice. Five weeks old, male albino mice were used as experimental animals in order to demonstrate the effect of *Ocimum basilicum*'s extract on learning and memory. Each male albino mouse was weighted and orally treated either with 100 mg/ ml solvent/ Kg body weight of *Ocimum basilicum* leaf extract or with commercially available saline solution (Otsuka, Pakistan) for 7 days. Behavioral observations were made by applying a series of neurological tests (Elevated plus maze, Light and dark box, Open field and Rota rod). Dose supplementation continued during neurological testing. It was observed that 100 mg/ ml solvent/ Kg body weight of leaf extract improves neuromuscular co-ordination and male albino mouse performance in open field, light dark box and during novel object test when compared with control group. We concluded that 100 mg/ ml solvent/ Kg body weight of leaf extract has potential to improve neuromuscular co-ordination, exploratory behavior, object recognition ability and transfer latency in male albino mice and can be safely administrated orally.

MOLECULAR IDENTIFICATION OF BACTERIAL PATHOGENS ISOLATED FROM HONEY (PROCESSED AND UNPROCESSED) AND THEIR INHIBITION BY MEDICINAL PLANTS

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In the present study, the bacterial population was investigated from five commercial honey products (processed honey) and five unpackaged honey products (unprocessed). Out of total 10 isolated pathogenic bacteria, only one was isolated from processed samples, while the remaining nine were isolated from unprocessed honey samples. These Gram negative and Gram positive bacterial contaminants were identified as *Bacillus cereus*, *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium septicum*, *Bacillus weihenstephanensis*, *Clostridium acetobutylicum*, *Proteus mirabilis*, *Myroides odoratimimus* which are common soil and water dwelling bacteria. Intrinsic human pathogens i.e. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were also identified. Optimum conditions for bacterial isolates were determined. The high number of pathogenic bacteria in unprocessed honey indicated secondary contaminations of honey by poor handling. Among the tested medicinal plants two bacterial strains (*B. cereus* and *B. anthracis*) was strongly inhibited by methanolic extract of *N. sativa* and one strain (*B. weihenstephanensis*) inhibited by *C. sinensis*. On the other hand *Aloe vera* and *Cinnamom umverum* had no effect on the growth of all tested pathogens. All tested bacterial pathogens were strongly inhibited by using Ofloxacin as compared to other tested antibiotics.

**ROLE OF OLIVE LEAVES AS ANTI-VIRAL AGENT IN NDV
INFECTED BROILER CHICKS**

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Antiviral effects of olive leaves (*Olea europaea*) in NDV infected broiler chicks were observed. Birds of positive control group were not supplemented with olive leaves. Birds of group D and E were infected with NDV on day 19th and 1g/kg and 2g/kg of the olive leaves were supplemented in feed of these broiler chicks groups, respectively. The sloughing of intestinal epithelium, lymphocytic infiltration in the laminae propria and hemorrhages in the muscularis mucosa of intestine were observed in the infected birds. Hemorrhages, sloughing and congestion were also observed in the proventriculus. There was Significant increase in the body weight and total Leucocyte count of broiler chicks in all the olive leaves supplemented groups as compared to control group. Packed cell volume (PCV) in all olive leaves treated groups was Significant decreased as compared to control group. The present study revealed that dietary supplementation with olive leaves has beneficial effects on the performance and body weight of the broiler chickens.

**BIO-AUTOGRAPHY, SPOT SCREENING, ANTIBACTERIAL ACTIVITY OF RUMEX
HASTATUS EXTRACTS AND ITS FOURIER TRANSFORM INFRARED AND
RAMAN SPECTROSCOPY**

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Human infectious diseases began by various pathogens including bacteria, fungi, viruses, parasites, and protozoans. These infectious agents controlled using synthetic drugs as well as natural sources. In current research antibacterial effect of *Rumex hastatus* was analyzed against seven clinical pathogenic bacteria such as *Escherichia coli*, *Serratia marcescens*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* through agar well diffusion method. Boiled extract was used for phytochemical screening, antioxidant potential, thin layer chromatography, bioautography, and spot screening. Genomic DNA was extracted to find the effect of *R. hastatus* on DNA synthesis inhibition. Antibacterial results revealed that diethyl ether extract showed the maximum inhibition of *S. pyogenes* (9.66±0.57 mm). Acetone and diethyl ether extracts showed moderate inhibition of *K. pneumoniae* (6.33±1.52 mm and 5.66±1.15 mm) and *S. aureus* (6.33±1.52 mm and 5.66±0.57 mm). Similarly, chloroform extracts indicated moderate inhibition of *S. pyogenes* (5.66±1.15 mm). Ethanol had low or even no effect on the growth of bacteria. Genomic DNA extraction also encouraged the antibacterial effect of *R. hastatus*. Various phytochemical constituents such as

ketoses, oligosaccharides, amino acids, amines, sugars, flavonoids, and antioxidant constituents were detected. TLC-Bioautography and spot screening results revealed the potential use of *R. hustatus* as an antibacterial agent. It was concluded that most the tested fractions appeared as an important source for the discovery of new antimicrobial drugs having the massive remedial potential to cure many infectious diseases with low or minimal side effects.

**THALASSAEMIA MANAGEMENT WITHOUT BLOOD TRANSFUSION T.S.H
INADEQUACY CAUSES D.V.T G.M**

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The commencement in D.V.T is phlebothrombosis which causes ischemia of the respective part of the organ of the body that result in grave situation of necrosis. This inflammation transforms the vein into thrombophlebitis. On the detachment of the clot risk of pulmonary embolism may be faced. Deep vein thrombosis(D.V.T) is created due to rather sluggish blood circulation in the arteries, that affects poor flow of the blood in the vein. This weak movement can be observed, while checking blood pressure, through stethoscope in the form of shallow pitched sound. This weak sound is the result of low leveled energy production of the body cells, also affecting heart muscles, by subnormal thyroid secretions or hypothyroidism; or other prevalent known factors. In our case study, on screening thyroid profile that the patient was hypothyroid, but without shooting of TSH indicated that adenohipophysis is not properly responding i.e. That its feed-back mechanism is not working properly, for achieving life-long cure, adenohipophysis should be targeted. Its treatment consists of two phases; the first is peaceful dissolution of thrombus and second is normalization of the physiology of adenohipophysis so that it may properly regulate thyroid gland that may restore normal cytophysiological activity of the body to maintain normal energy production. Thus heart can pump blood with adequate force so that venous clot may not be re-formed. Homeopathy provides proper means in achieving complete cure. First, for the dissolution of the clump or thrombus from the blood is essential to restore normal circulation of affected organ of the patient, and to maintain proper cytophysiology in restoring normal hormonal level and proper force in the circulatory system. It will not only safe from the hazards of thrombosis but also provide a lot of other health advantages. For dissolving thrombosis of the vein for restoring smooth flow of the blood; we have *Arnica montana*; and *Kalmia* disassembles the thrombus; *Crataegus oxyacantha* provides force to the cardiac muscles; *Eletaria cardamom* dilates artery's lumen to allow enough blood flow in the vessels. *Fucus vasiculosus* provides organic molecule of iodine to the body in enhancing iodine level for the production of T3, T4 from the thyroid gland without the help of extra TSH. In obtaining life-long security and safety of the patient we have to achieve permanent normalization of physiology of thyroid gland. This was elaborately discussed in 36th congress of Zoology. In present case study I have met a patient of D.V.T with hypothyroidism but without shooting up of TSH. Indicates that his adenohipophysis is not properly working for this propose we have *Pituitrinum* along with its helping remedies like *Baryta carb* and *Graphites*

IMMUNOTOXICOLOGICAL EFFECTS OF CARBOFURAN AND ITS AMELIORATION WITH STAR ANISE (*ILLICIUM VERUM*) IN BROILER

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The objective of this study was to investigate the immuno modulatory and ameliorative effects of star anise against immunotoxic effects of carbofuran in broilers. One day-old chicks were divided into 6 groups (15birds/group) and fed ad-libitum feed for 4 days. At 5th day we gave them carbofuran by crop tube feeding method (6mg/kg and 10mg/kg) alone and in combination with star anise (6g/kg) and star anise (6g/kg) alone for 42 days. Immunological responses, including antibody formation against sheep red blood cells (7 and 14 days after both primary and booster injection), lymphoproliferative responses to avian tuberculin (30 day of age), and mononuclear phagocytic system function (by carbon clearance assay test) at 42 days of age were measured. Results showed that star anise alone and in combination with carbofuran ameliorated the immunotoxic effects of carbofuran by (6mg/kg and 10mg/kg). The results of the present study showed that star anise had an ability to ameliorate carbofuran induced toxicity in broilers.

ANTIOXIDANT ACTIVITY OF *NIGELLA SATIVA* SEEDS EXTRACT AND ITS USE FOR CRYOPRESERVATION OF NILI RAVI BUFFALO BULL SPERMATOZOA

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Nigella sativa (Black seed) has been used for therapeutic purpose for eras; however, its antioxidant potential for cryopreservation of buffalo semen has not been studied. The objective was to evaluate the free radical scavenging activity of *Nigella sativa* seed's aqueous extract, and its efficiency for cryopreservation of Nili Ravi buffalo bull spermatozoa. *Nigella sativa* seeds (10g) were manually crushed, powdered and mixed with 50ml distilled water. The suspension was vortexed for 15-20 minutes, filtered and sterilized to prepare aqueous extract. The free radical scavenging activity of the extract was evaluated by DPPH assay. The *Nigella sativa* extract concentrations at 1%, 2%, 3%, 4% and 5% showed an increased pattern of free radical scavenging activity (%) and 4% level was EC₅₀ value of the *Nigella sativa* extract. To assess in vitro dose tolerability of the *Nigella sativa* extract for buffalo bull sperm, semen was diluted in sodium citrate buffer and sperm motility was evaluated. *Nigella sativa* extract at all levels was non-toxic to buffalo sperm; rather, the sperm motility was increased at 1%, 1.5%, 2%, 3% and 4% of extract. To evaluate the efficiency of *Nigella sativa* extract for cryopreservation of buffalo semen, three Nili Ravi buffalo bulls kept at Semen Production Unit (SPU) Qadirabad, Sahiwal were used to collect semen for three consecutive weeks (replicates). Ejaculates that qualified the minimum criteria were pooled and cryopreserved with tris citric egg yolk extenders containing *Nigella sativa* extract at

1%, 2%, 3%, 4% and 5% (vol/vol). Sperm motility and plasmalemma integrity was evaluated after dilution and after cooling, while sperm motility, plasma membrane integrity, viability and DNA integrity were evaluated after freeze-thawing. After dilution, sperm motility was higher ($P < 0.05$) in extenders supplemented with *Nigella sativa* extract at 1%, 2%, 3% and 4%, while, PMI was higher ($P < 0.05$) in extenders with 4% and 5% extract compared to control. Post cooling sperm motility and PMI was higher ($P < 0.05$) in extender supplemented with 4% *Nigella sativa* extract compared to control. At post thaw stage, sperm progressive motility and DNA integrity was significantly ($P < 0.05$) improved in extenders supplemented with *Nigella sativa* extract at all levels, while, sperm PMI and viability was improved ($P < 0.05$) in extenders with 4% and 5% extract compared to control. It is concluded that *Nigella sativa* aqueous extract possesses antioxidant activity and its supplementation at 4 - 5% in tris citric acid extender improved sperm quality parameters at all stages of semen cryopreservation in Nili Ravi buffalo.

EFFECT OF ETHANOLIC EXTRACT OF *CORIANDRUM SATIVUM* L. LEAVES AND STEMS ON REPRODUCTIVE ORGANS OF MALE ALBINO RATS.

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Coriander (*Coriandrum sativum* L.) belonging to the family Apiceae is a small herb. It is considered as a medicinal plant due to the anti-hyperglycemic, anti-hyperlipidemic, antiproliferative, hypotensive and digestive stimulant properties. The present study investigated the effect of ethanolic extract of *Coriandrum sativum* L. on male reproduction using the laboratory rat as a model animal. Ethanolic extract of *Coriandrum sativum* L. leaves and stems was administered orally at low and high doses in rats for acute treatment. Toward the end of experiment, blood was drawn from the heart to determine the serum testosterone (ng/ml), while the reproductive organs viz., epididymis, seminal vesicle and prostate were used to determine the sperm count (10^6 /ml), status of oxidative (absorbance), antioxidant enzymes (U/min) and examination and histological. Serum testosterone (ng/ml) and epididymal sperm count (10^6 /ml) decreased significantly ($p < 0.001$), with a parallel decrease ($p < 0.05$) in testicular SOD (unit/min), POD (unit/min) ($p < 0.05$), CAT (unit/min) ($p < 0.001$) and GSH (unit/min) ($p < 0.05$) activities, whereas significant increase was noticeable in ROS (absorbance) ($p < 0.05$) activity in the testes. Histologically, seminiferous tubular lumen widened spermatogonia and Leydig cells appeared compromised. Epididymis revealed irregular epithelia cells, decreased sperm content in the lumen, inward folding of cells, while in the seminal vesicle and prostate, lamina propria was thickened and folded intact ruptured epithelial cells were the outcome. Acute administration of low dose (2000 mg/kg) of *Coriandrum sativum* L. led to a significant decrease of sperm count (** $p < 0.001$) in the epididymis; significant increase in ROS (** $p < 0.001$) and CAT (* $p < 0.05$) in the testes. The high dose (4000 mg/kg) showed significant decrease in serum testosterone level (* $p < 0.05$), sperm count in epididymis and POD (* $p < 0.05$) in the testes. Histopathological alterations were observed in testes and male accessory reproductive organs in acute treated groups. Acute low dose (2000 mg/kg) administration of *Coriandrum sativum* L. extract caused a significant increase in Cu concentration ($\mu\text{g/g}$) in testes (* $p < 0.05$), epididymis (** $p < 0.001$), Ni ($\mu\text{g/g}$) in testis and epididymis (* $p < 0.05$), Ca ($\mu\text{g/g}$) in blood, testis, epididymis, seminal vesicle (* $p < 0.05$) and prostate (** $p < 0.001$), K ($\mu\text{g/g}$) in blood, testis, epididymis, seminal vesicles and prostate (* $p < 0.05$), Na ($\mu\text{g/g}$) in blood (* $p < 0.05$). Whereas, the administration of same dose showed significant decrease of Zn ($\mu\text{g/g}$) and Mg concentrations

($\mu\text{g/g}$) in testes ($*p<0.05$). The high dose (4000mg/kg) showed significant increase of Ni concentration ($\mu\text{g/g}$) in prostate ($*p<0.05$), Ca concentration ($\mu\text{g/g}$) in testes ($*p>0.05$), K ($\mu\text{g/g}$) in epididymis, seminal vesicle and prostate ($*p<0.05$). Whereas, the administration of same dose showed significant decrease of Cu ($\mu\text{g/g}$) and Mn ($\mu\text{g/g}$) in prostate ($*p<0.05$), Ni ($\mu\text{g/g}$) in seminal vesicle ($*p<0.05$), Mg ($\mu\text{g/g}$) in blood ($**p<0.001$), testis, epididymis, seminal vesicle and prostate ($*p<0.05$), Na ($\mu\text{g/g}$) in seminal vesicle and prostate ($*p<0.05$), Cr ($\mu\text{g/g}$) in testis ($*p<0.05$) and in male accessory glands ($**p<0.001$). The current study concludes that use of coriander in large quantities may have inhibitory effect on reproduction but implicating the anti-fertility effects.

SUB-ACUTE AND SUB-CHRONIC EFFECTS OF *NIGELLA SATIVA* OIL ON MICRO- AND MACRO-ELEMENTS OF SELECTED TISSUES IN LABORATORY RATS Ibrar

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Medicinal importance of several plant species has been proven in traditional medicine. Among them is a small herb called *Nigella sativa* of the family Ranunculaceae. A large number of studies have shown that *Nigella sativa* possess activities like diuretic, anthelmintics, analgesics, antihypertensive, and immunomodulatory, antidiabetic, anticancer antimicrobial, and antioxidant properties, renal protective, gastro protective, anti-inflammatory, bronchodilator, hepatoprotective, and spasmolytic, but its role in the body's elemental concentrations has not been demonstrated. Macro-elements Ca, Mg, K, and Na are the integrative elements of bones, genetic materials, membranes, different enzymes and proteins. Trace elements including Fe, Zn, Cu, Cr, Mn and Ni control cellular functions at molecular, chemical and biological level, so they are very important in optimal concentrations. Any alteration in their concentrations can create imbalance in physiological conditions and hence diseases. Presently, oil of *Nigella sativa* was administered orally at doses of 120 ml/kg and 60ml/kg during subacute and sub-chronic treatments. Control group was administered with distilled water. A total of thirty six male rats were used. Each group consisted of six rats. Digests of the selected tissues were prepared in nitric acid. The concentration of each metal was detected through atomic absorption spectrophotometer. Data are described as mean \pm SEM. For dose treated groups and control groups comparison one-way analysis of variance (ANOVA) was used. For statistical significant difference, the $P<0.05$ was considered as a standard. Sub-acute high dose administration of *Nigella sativa* oil caused a significant increase in Zn level of brain, blood, lungs and Cu in blood ($**p<0.008$). Significant decrease ($***p=0.0001$) occurred in Cr and Mn in all the tissues. A decrease also occurred in Ni in brain, blood, heart, muscle, lungs, stomach, kidney ($***p=0.0001$), and Mg in heart, stomach, kidney, Fe in liver ($***p=0.0001$), Ca in brain and K in heart and kidney. Low dose significantly decreased Cr concentration ($***p=0.0001$) in blood, muscle, skin, liver, stomach, intestine, Mn in skin, lungs, stomach, Na in stomach and Ca in brain, liver, stomach and intestine. Sub-chronic administration of high dose of *Nigella sativa* significantly decreased Fe level in brain, blood, muscle, skin, lungs, liver, stomach, intestine, kidney ($***p=0.0001$), Ni in heart, skin, kidney ($***p=0.000$), Na level in blood ($**p<0.002$), Ca level in lungs ($**p<0.001$), while similar dose led to a significant increase in Zn level in liver ($***p=0.0001$), Cu level in blood ($***p=0.0001$). Administration of low dose significantly decreased Fe level in muscle ($***p=0.0001$) and same dose increased Cu level in intestine ($***p=0.0001$). It is concluded from the present study that different doses and dose duration of *Nigella sativa* significantly alters mineral concentrations in body tissues of rats but the cellular mechanisms causing this change needs further investigations.

CHIKUNGUNYA VIRUS AND HOMEOPATHIC MEDICINES

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The term “Chikungunya” is derived from African language means “to be bent over” it is also called “buka buka” in Congo, commonly called as Chikungunya virus (CHIKV) and Chikungunya fever (CHIKF). This is an arbovirus belongs to the family Togaviridae, being an arbovirus, it maintained in the environment between humans or other animals and the vector mosquitoes. During epidemics, humans usually serve as major reservoirs. It was first reported in African Village Swahili of Newala district of Tanzania in 1953. Chikungunya fever has also been accounted from African Countries, Western Countries, Tropical and Temperate Zone and South East Asian Countries. In Dehli, City of India, the outbreak of Chikungunya was reported in 2016. The Chikungunya virus is transmitted to humans through day-biting mosquitoes *Aedes aegypti* or *Aedes albopictus*. These mosquitoes are also involved in transmission of Dengue fever and Dengue Hemorrhage fever. Prevalence based on quite a lot of socioeconomic factors, vulnerability of humans and mosquitoes to the virus and role of mosquitoes for the transmission of disease. The symptoms of Chikungunya infection are high fever and severe joint pains with swelling and stiffness especially in elbow, knee, wrist and ankle joints also rashes on the skin shows red or purple discoloration on the skin due to bleeding from vessels under skin, other symptoms including vomiting, nausea and headache. The fever is last 3 to 4 days but joints pains may continue for week to years. There is no any vaccine is available. Recently Malir district and adjoining areas of Karachi city was highly affected with Chikungunya virus. The Homeopathic medicines are very effective and natural treatment with negative side effects.

Arnica: This Remarkable Homeopathic remedy is the best for Chikungunya infection especially in rashes and discoloration on skin due to bleeding from vessels under skin or petechial spots. Unbearable joints pain with swelling and stiffness. Pain in whole body and slight touch increases the pain and coldness of hand and feet. **Rhus Tox:** The Best Homeopathic Medicines for Chikungunya infection, especially in Fever with joints pain, the joints pain increases during the rest and feel relief during the motion. Joints pain with swelling and stiffness, patient feel restless after going to bed and want to change position. Worse backache and chill during fever. **Bryonia:** The most excellent Homeopathic remedy for Chikungunya infection with extreme joint pains. The joints show inflammatory symptoms like heat, swelling and pain. Patient gets relief in pain during rest and worsens during motion including dryness of mouth and thirst. **Phosphoric Acid:** The top remedy for weakness during the Chikungunya infection, during and after the weakness of fever, when patient feels mentally and physically flaw.

**CLONING, EXPRESSION AND PURIFICATION OF XYLANASE GENE
FROM ANOXYBACILLUS RUPIENSIS**

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Xylanases are considered as one of the principal enzymes used in industry for the hydrolysis of a heteropolysaccharide, xylan. The present study refers to the isolation of

xylanase encoding gene from *Anoxybacillus rupienesis*. The amplified xylanase gene from *Anoxybacillus rupienesis* was cloned in pTZ57R/T cloning vector and transformed in *E. coli* DH5 α . For the purpose of expression, cells of *E. coli* BL21 CodonPlus were used for transformation while pET-22a(+) vector was used for sub cloning . The expression was then confirmed by running on 12% SDS-PAGE. The crude enzyme obtained was then purified by precipitating it with ammonium sulphate salt and further purification was done using gel filtration chromatography technique. Ammonium sulphate precipitation resulted in 70.0 U/mg of specific activity in cell lysate while supernatant showed 72.5 U/mg of specific activity. Gel filtration chromatography resulted in specific activity of 94.44 U/mg and 86.36 U/mg in cell lysate and supernatant respectively. The purified enzyme was then characterized for optimization of temperature and pH. Thermostability and stability of pH was also calculated. The optimum temperature at which xylanase showed highest activity was 70°C but thermostability was obtained at 65°C. The optimum pH at which xylanase gave highest activity was 6.0 and was noticed to be stable at same pH. Effect of different ions was also examined on xylanase activity. Some ions such as Ca⁺² and Mg⁺² resulted in 2.5 and 2.3 folds increased activity, respectively. While the activity of xylanase enzyme decreased in the presence of Triton-X100, EDTA and cadmium.

SECTION – I I**PESTS AND PEST CONTROL****INCIDENCE OF *CHILO INFUSCATELUS* SENELLEN (PYRALIDAE: LEPIDOPTERA)
ON DIFERENT VARIETYS OF SUGARCANE IN BADIN**

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Sugarcane stem borer, *Chilo infuscatelus* Snellen, 1890, is an important and destructive insect that attacking sugarcane crop every year (Khanzada, 1995). *Chilo infuscatelus* damages the crop during the shoot stag as young larvae first feed on the out leaves of sugarcane plants. During the present study, 122 specimens were collected from different localities of district Badin, Sindh, which is sorted out into family Crambidae, and single species i.e. *Chilo infuscatelus*, while other material in the process of identification.

**EVALUATION OF ESSENTIAL OILS FROM SOME INDIGENOUS PLANTS AGAINST
RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM***SYEDA AZRA TARIQ,¹ MUHAMMAD FARHANULLAH KHAN,² AMJAD PERVEZ,¹
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Insecticides based on plant essential oils are ecologically acceptable substitute for pest control. Owing to their volatility, the oils and their essentials are environmentally degradable and relatively nontoxic to mammals. Aiming at the use of plant oils for safe, economical and environment friendly storage of grains, rodent control baits and food stuffs a study was carried out to evaluate comparative toxicological effect of five indigenous plant oils, for their efficacy) against red flour beetle, *Tribolium castaneum*. For the purpose, five essential oils of the plants viz, *Azadirachta indica* (neem), *Valeriana officinalis* (valerian), *Acorus calamus* (sweet flag), *Curcuma longa* (turmeric) and *Saussurea lappa* (costus) were tested to estimate their toxicity and efficacy as insect growth regulator (IGR). The plant oils were tested in three (0.1%, 0.05% and 0.025%) doses in comparison with control. For the purpose, wheat flour was treated with the doses for all the plant oils. Fifty grams of treated and untreated (control) wheat flour was kept in glass jars covered with muslin cloth. All trials were replicated five times, under the same temperature and humidity. No significant mortality was observed by the oils except neem oil causing 68.75% mortality at 0.05% concentration to adult *Tribolium castaneum* after 72 hours. All plant oils reduced growth of the insect during metamorphic stages. Maximum larval inhibition (88.35%) was observed by valerian oil at 0.1% concentration; whereas, maximum pupal inhibition (77.77%) was observed by costus oil at 0.05% concentration. Minimum larval inhibition (4.09%) was observed by neem oil at 0.025% concentration; whereas, minimum pupal inhibition (6.83%) was observed by neem oil at 0.05% concentration. All the oils showed excellent effects against the emergence of adults however maximum adult inhibition (98.55%)

was observed by neem oil at 0.1% concentration; whereas minimum adult inhibition (84.52%) was observed by valerian oil at 0.05. In view of determination of plant based management of grain pests, the findings of the study may be an addition for IPM models for the end-users.

ELECTROPHORETIC ANALYSIS OF *TRIBOLIUM CASTANEUM* AFTER COMBINED PESTICIDAL EFFECT OF *BACILLUS THURINGIENSIS* AND NATURAL PLANT PRODUCTS

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In Pakistan, there are many plants which contain insecticidal agents. In order to manage the loss of stored grains and other agricultural products from insect infestation, many plant products have been evaluated for their toxic properties against different stored grain pests. The red flour beetle; *Tribolium castaneum* has a long association with human stored food products as pest. *Bacillus thuringiensis* (*B.t.*) is spore forming aerobic, Gram-positive bacteria that produces insecticidal crystal (cry) proteins. Cry proteins produced by *B.t.* are toxic and an alternative to synthetic insecticides. This bacterium occurs naturally in the environment and has been used as a biological pesticide for more than 50 years and grown on its specific media by microbial techniques. Cinnamon (*Cinnamomum aromaticum*), turmeric powder (*Curcuma longa*) and Black onion seed (*Nigella sativa*) are used as insect repellent. An individual and combined biotoxicity assay of these compounds with *B.t.* was performed in which the 2nd instar larvae and adults of *T. castaneum* were used as target pest to check the effectiveness of three natural compounds individually and in combination. The LC₅₀ values of Cinnamon and Black onion seeds powder were 0.4g/g, 0.35 g/g against the larvae of *T. castaneum*. Combined bioassay of these compounds with 3 isolates of *B.t.* was done and synergistic effect with high mortality rate of *Tribolium castaneum* was observed. The Biotoxicity assay data was analyzed by probit analysis programme. Then electrophoretic analysis of experimental and control was also performed which was composed of DNA, RNA and protein isolation and then their complete study and visualization on agarose gel electrophoresis and polyacrylamide gel electrophoresis (PAGE) respectively. Electrophoretic analysis proved significant degradation of mRNA and hence protein structure and function or expression.

POTENTIAL SYNERGIES OF SOIL MACRO-FAUNA AMONG TOMATO (*SOLANUM LYCOPERSICUM* L.) AND CAULIFLOWER (*BRASSICA OLERACEA* L. VAR. *BOTRYTIS*) FIELDS

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During the present study, among tomato fields, total 77 species were recorded belonging to 13 orders, 38 families and 62 genera; whereas among cauliflower fields, total 41 species were counted pertaining to 10 orders, 23 families and 35 genera. Among both fields, total 1757

specimens were collected during entire sampling (07 sampling from each category) and maximum population was recorded from tomato fields 58.05% (N = 1020) and least population was recorded from cauliflower fields i.e. 41.95% (N = 737). Wherein the population means per sampling was also calculated along with standard deviation (SD) (Table 03). In case of tomato fields, maximum population was recorded during 7th sampling (252±75.16), followed by 173 ± 19.30 (6th sampling), 154 ± 5.86 (4th sampling) and so on. While, least values were recorded during 1st sampling (64±57.78). Whereas, species abundance was recorded utmost during 4th and 6th sampling (44 species) at temperature and humidity of 17 °C, 18 °C and 80%, 71%, respectively. However, least species abundance was recorded during 1st sampling i.e. 13 species at 13°C (temperature) and 50% (humidity). In case of cauliflower fields, maximum population was recorded during 6th sampling (190 ±59.90), followed by 167± 43.64 (4th sampling), 135± 21.01(5th sampling) and so on. While, least value was recorded during 2nd sampling (27±55.36); whereas species abundance was recorded utmost in 6th and 4th sampling (15 and 11 species, respectively) at temperature and humidity 11°C, 84% and 21 °C, 81%, respectively. However, least species abundance was recorded during 3rd sampling i.e. 05 species at 26°C temperature and 90% humidity. From tomato control, maximum relative abundance 37.45% (N = 382) was recorded for *Succinea* spp. as an extraordinary contributing species. In case of cauliflower control maximum relative abundance 57.12% (N =421) was recorded for *Succinea* spp. From total of (44) recorded families, 37 were recorded from tomato control and among them, extra ordinary relative abundance (47.84%; N = 488) was recorded for Succineidae and then maximum relative abundance was recorded for Formicidae family (14.61 %: N = 149), Porcellionidae (5.10 %; N = 52), Lycosidae (4.31 %; N =44), Trichoniscidae (3.53%; N = 36), Subulinidae (2.06 %; N = 21), Anisolabididae (1.96 %; N = 20), Tenebrionidae, Lumbricidae (1.86; N = 19), Gryllidae (1.57%; N = 16), Trachelipodidae (1.47%; N = 15), Platyarthridae, Cimicidae (1.37%; N = 14), Planorbidae, Cylisticidae, Vespidae (1.27%; N = 13), Labiduridae (0.98%; N = 10), Agelenidae (0.88%; N = 09), Coccinellidae, Hygromiidae (0.69%; N = 07), and Scarabaeidae, Dermestidae, Cryptopidae (0.59%; N = 06). From total of 44 recorded families, 23 were recorded from cauliflower control and among them, relatively higher abundance (66.08%; N = 487) was recorded for Succineidae family. Thereafter, relative abundance was recorded for Formicidae (11.40%; N =84), Enidae (6.11%; N = 45), Gryllidae (2.44%; N = 18), Coccinellidae, Platyarthridae (2.04%; N = 15), Oniscidae (1.90%; N = 14), Lumbricidae (1.36%; N = 10), Chrysomelidae (1.09%; N = 08), and Acrididae, Agelenidae (0.59 %; N = 06). Diversity (H') was recorded maximum among tomato control (1.6280) and least was recorded among cauliflower control (1.0055). Dominance was recorded maximum from tomato control (0.4589) and least from cauliflower control (0.6493).

**COMPARATIVE STUDY RELATED TO PREDATORY PREFERENCE OF
MELANOSTOMA SCALARE (APHIDOPHAGUS HOVERFLY: SYRPHIDAE) ON FOUR
APHID SPECIES IN PUNJAB, PAKISTAN.**

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Aphid is major cause of annual reduction in valuable cereal crops; one of them is wheat and *Brassica* crops in Punjab, Pakistan. An ideal natural enemy is one that consumes sufficient preys at the right time to maintain a pest population below the economic injury threshold for the crop

considered. *Melanostoma scalare* is one of aphidophagous syrphids, belongs to subfamily syrphinae. Predatory preference of *Melanostoma scalare* was determined by providing them four species of aphid's *Brevicoryne brassicae*, *Myzus persicae*, *Schizaphis graminum* and *Rhopalosiphum padi* for its consumption. Significant difference ($P=0.00$) in predatory efficiency of *M. scalare* larvae on four aphid species was found. *S. graminum* was most preferred prey species with 504 ± 22.9 specimens consumed by larvae of *Melanostoma scalare* followed by 495 ± 48.6 of *M. persicae*, 424 ± 24.6 *B. brassicae* and relatively less number of *R. padi* as 396 ± 10.03 specimens were consumed. Consumption in different larval instars was also measured and highest consumption during 3rd instar larvae was recorded on *M. persicae* 336 ± 31.5 followed by *S. graminum* 324 ± 8.6 and relatively less aphid consumption was on *B. brassicae* 298.7 ± 18.8 and *R. padi* 282.3 ± 8.34 respectively. Larvae of *M. scalare* consumed varied number of aphids in different days of three instars. Significance difference was found in its average per-day consumption. It was greater on *S. graminum* 42 ± 1.91 and minimum of 33 ± 0.93 specimens of *R. Padi* were consumed per day. While maximum survival 79% of *M. scalare* larvae was recorded when fed on *R. padi* was followed by 77% on *B. brassicae*, 73% on *S. graminum* and 69% on *M. persicae*. Predatory preference of *M. scalare* will be helpful for biological control of these aphid species.

**SCELIO AS BIO-CONTROL AGENTS FOR EMBRYONIC STAGES OF GRASSHOPPER
(ACRIDADAE: ORTHOPTERA)**

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The genus *Scelio* is a cosmopolitan and spacious group of parasitoids of the eggs of short-horned grasshoppers (Orthoptera: Acrididae). A number of these hosts are important pests, including plague locusts of the genus *Schistocerca* important biological control agents. 02 important species viz: *Scelio aegypticaus* Priesner 1951 and *Scelio hieroglyphi* Timberlake occurring in Pakistan their identification key along with description and photographs was also provided. During the present study a total of 2710 adult and nymphs of acridid was reported from different climatic zones of Pakistan. Nymphs were collected greater in numbers with 79.81% while adults ration was 9.8% for male & 10.33% for female respectively. Beside this, greater No. of egg-pods was collected from shrubs, herbs and grasses with 38.84% followed by 26.14% from paddy field. Further, it was also noticed that less percentage of hatching was obtained from egg-pods which were partially or completely parasitized by *Scelio*. This is first ever effort is being carried out from this region. Present study recommends that as the *Scelio* spp. cause major destruction in embryonic stages of acridadae so it could be implemented as bio-control agents at large scale.

**SEASONAL MONITORING OF TWO DIFFERENT SPECIES OF *BACTROCERA*
THROUGH INSTALLATION OF METHYL EUGENOL TRAPS IN GUAVA
ORCHARD MIRPURKHAS, SINDH**

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Bactrocera species are pests of many crops having dynamic status and accountable for the small production and low quality of fruits in Pakistan. 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 different highest i-e surface, 1 meter, 2 meters and 3 meters all were installed at Sindh Horticulture Research Institute guava orchard, Mirpurkhas with 1512.19±15.03, 1366.34±13.48, 1595.63±16.91 and 1360.73±14.05 trap catches while *B. dorsalis* catches in methyl eugenol pheromone traps with 17.13±0.10, 16.10±0.09, 18.35±0.10 and 15.62±0.10 installed at SHRI guava orchard, Mirpurkhas at the same highest of *B. zonata*. Beside this, these two species infestation was highest when the methyl eugenol pheromone traps were installed at 2m height compare to traps installed at 1m and 3m height could not catch *B. zonata* and *B. dorsalis* more than surface installed traps.

**DETERMINATION OF HOST PREFERENCE OF *PIERIS BRASSICAE*
(LEPIDOPTERA:PIERIDAE) ON FOUR SELECTED CROPS.**

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Major constraints to cereal production are stem borers and are responsible for 5-73% loss in different agro-ecosystems. Among them the cabbage butterfly *Pieris brassicae* is one of the most destructive insect pests, damaging various crops at all the stages ranging from seedling through flowering. The young caterpillars feed gregariously on leaves, resulting in defoliation of plants. Host plant preference of this pest was studied with respect to: the (1) consumption rates of their larvae on four host plants viz. brassica, cabbage, cauliflower and turnip leaves, and (2) effects of consumption rate on their pupal weights and percent adult emergence. To check these parameters *P. brassicae* larvae were fed on all of above mentioned four crops in separate cages. Along with these cages similar weighted crop (food) was placed in separate cages to check water loss. After intervals food consumed was calculated by getting weight of left over feed. Larval duration was 13 and 18 days when larvae were fed on turnip and brassica respectively. Total consumption and pupal weight of *P. brassicae* on different host plants was also checked. Brassica was the most preferred diet of *P. brassicae* and it consumed 21.36±0.72g of brassica leaves during its entire larval duration. *P. brassicae* showed least consumption on turnip 8.22± 0.60g. Pupal weight of *P. brassicae* was also maximum (0.61±0.01g) when caterpillars were fed with brassica leaves followed by cauliflower (0.52±0.02g), cabbage (0.49±0.01g) and, finally, turnip (0.40± 0.00g). Thus, pupal weight was directly correlated with the food consumption. A strong correlation among pupal weight and percent adult emergence of *P.*

brassicae was also observed. Percent adult emergence was maximum (90) along with pupal weight on brassica, followed by cauliflower (80), cabbage (80) and turnip (65). Host-plant relationships are necessary for development of efficient strategies for lepidopteran population control.

**IN VITRO CONTROL OF LEPIDOPTERAN CATERpillARS BY
BRACONID LARVAL PARASITIS.**

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Lepidopteran caterpillars are disastrous pest of many agricultural crops. They render economic losses to human being. Use of chemicals has been very effective solution to handle such types of problems. No doubt chemicals are very effective, fast in action, economical and can be applied in all types of habitats but these have disastrous effects on ecosystem health and natural enemies. The Braconidae (Hymenoptera) is among one of the largest families which is being used successfully in biological control programs worldwide. Most of the members of family Braconidae are endoparasitoids of holometabolous insects like *Pieris brassicae*, *Helicoverpa armigera*, *Trichoplusia ni* and *Spodoptera exigua*. Among braconidae Microgastrinae is the most abundant and diverse group which are parasitoids of lepidopteran caterpillars and *Cotesia* is the largest genus of Microgastrinae. Three of the species of braconid larval parasitoids (*Cotesia vestalis*, *C. flavipes*, and *C. plutella*) were selected on the basis of their relative abundance, these were subjected to study their life cycles while providing 4 lepidopteran species viz *P. brassicae*, *H. armigera*, *T. ni* and *S. exigua* (five specimens of a single species of different instars randomly in a cage) to check their host suitability and preference. *C. plutella* preferred none of the host species while both *C. vestalis* and *C. flavipes* used *P. brassicae* as host and not all the three remaining. Both of these species are endoparasitoids. Average incubation and larval duration was recorded to be 10.33 ± 0.47 days for *C. vestalis* while it increased to 11.67 ± 0.41 days for *C. flavipes*. After completion of larval development larvae of both of the species ruptured caterpillar from dorsal side and attached to the surface of cage and spun a cocoon around them. Ten cocoons were found in each of the trials of *C. flavipes* while 14 cocoons were spun by *C. vestalis* larvae. Pupal duration was more for *C. flavipes* (8.00 ± 0.82 days), while it was 6.33 ± 0.94 days for *C. vestalis*. Adult life span was only 2.00 ± 0.45 days for *C. vestalis* and 2.50 ± 0.47 days for *C. flavipes*. Overall duration in days for *C. vestalis* was recorded to be 18.66 ± 6.67 days and for that of *C. flavipes* 22.17 ± 8.01 . Such type of studies are very important in designing biological control programs.

**ENTOMOPATHOGENIC FUNGI AND *BACILLUS THURINGIENSIS* IMPACT
ASSESSMENT ON ARTHROPOD PESTS OF RICE**

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The rice crop is one of the most vital cereals. It provides food for nearly half of the world's population. It is attacked by numbers of insect pests which cause serious damage to the crop.

Generally synthetic pesticides are used to control insect pests but it is elevated that these pesticides have serious impacts on the environment and human health. Keeping in view, the present study was conducted to determine the efficacy of microbial pesticides against insect pests of rice. The entomopathogenic fungi and *bacillus* species were used for experimentation. The rice plants were grown in pots and in the field in Young wala research area of Department of Entomology, University of Agriculture Faisalabad. The experiments were done in pots with three experiments as well as in the field by using the entomopathogens alone and in combinations. At the emergence of plants different concentration of entomopathogens were applied against rice pests. After the application the mortality data in pots was collected after 24, 48 and 72 hours and in field the mortality data was collected after 48 and 72 hours. The field data was collected after every three days of application for the evaluation of long term efficacy of entomopathogens until the second application. All the data was analyzed by statistical software Statistix 8.1 using Tukey HSD for mean at 5% of level of confidence. The highest mortality of rice leaffolder and sugarcane pyrilla is caused by pure formulation in experiment No.1. The fungal isolate alone produced 45.62 and 81.73% mortality of Rice leaffolder after 48 and 72 hours respectively after the application of pure formulation (experiment No. 1). *Bacillus thuringiensis* alone produced 45.69 and 81.98% mortality of rice leaf folder after 48 and 72 hours respectively in experiment No. 1 while the combination of both fungal and bacterial pure formulation produced 75.23 and 89.93% mortality of rice leaffolder after 48 and 72 hours respectively. Pure formulation of fungus also caused the mortality of Sugarcane pyrilla and produced 42.93 and 64.40% mortality after 48 and 72 hours while the pure formulation of bacteria alone produced 66.26 and 76.95% of mortality. The combinations of both formulations produced 72.96 and 82.59% of mortality after 48 and 72 hours respectively. The highest mortality produced by *Bacillus thuringiensis* alone in pots experiment is 47.19, 81.54 and 90.29% after 24, 48 and 72 respectively, while *Beauveria bassiana* produced 45.64, 62.39 and 79.44% mortality after 24,48 and 72 hours of application. The combination of both formulations provided 52.64, 90.31 and 90.39% of mortality after 24, 48 and 72 hours. So it is concluded that microbial pesticides can be used as potential biocontrol agent against insect pests of rice with no harmful effect on our ecosystem.

ESTIMATION OF LIFE CYCLE OF *PIERIS BRASSICAE* (LEPIDOPTERA:PIERIDAE) ON FOUR SELECTED HOST PLANTS

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Host plants play a vital role in establishment of colonies of polyphagous insects and their outbreaks. While presence of an insect on any crop plant is not an indication of the crop as host of particular insect. To prove a plant as host for a specific insect, the insect must complete its whole life cycle on that particular host plant. Host-plant relationships are necessary for development of efficient strategies for lepidopteran population control. These require studies on their habitat distribution and mode of damage to different crops. Therefore, developmental responses *Pieris brassicae*, was studied on four selected crops i.e. brassica, cabbage, cauliflower and turnip leaves. The average duration of larvae, pupae and adults along their body lengths, percent emergence and longevity of adult survival were recorded for above mentioned pest. Maximum of (17.67±0.47)

days larval duration was recorded when caterpillars were fed upon brassica with mean body length of (45.45 ± 0.05) mm and significantly less duration (13.00 ± 0.00 days and 35.20 ± 0.22) mm length ($P < 0.00$) was observed while feeding on turnip. Mean pupal duration of this species was found to be maximum of (11.00 ± 0.82) days for turnip with an average length of 18.30 ± 0.47 mm. Significantly least pupal duration ($p < 0.05$) was recorded on brassica crop of 6.67 ± 0.47 days, but in this case pupae attained maximum length of 20.00 ± 0.82 mm. For larvae which fed on brassica, the adults attained a maximum body length of 45.45 ± 0.05 mm. Adult survival was also greater of 6.67 ± 0.47 days for same crop, followed by 4.67 ± 0.46 days both for cabbage and cauliflower and significantly less ($p < 0.05$.) 3.00 ± 0.00 days when *P. brassicae* larvae fed on turnip. 90% adult emergence was recorded for brassica, 80% for both crops cabbage and cauliflower and 65% adult emergence was observed for Turnip.

ESTIMATION OF LIFE CYCLE OF *SPODOPTERA EXIGUA* (LEPIDOPTERA: NOCTUIDAE) ON FOUR SELECTED HOST PLANTS.

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Spodoptera exigua (Lepidoptera: Noctuidae) is a known pest of crops specifically vegetables throughout world especially in subtropical and temperate regions. Host plant may play a role in effecting different traits of phytophagous pests, such as in regulating pest populations. It can also affect body size, which in turn affects survival, life span and fecundity. Host-plant relationships are necessary for development of efficient strategies for lepidopteran population control. These require studies on their habitat distribution and mode of damage to different crops. Therefore, developmental responses of *Spodoptera exigua* was studied on four selected crops i.e. brassica, cabbage, cauliflower and turnip leaves. The average duration of larvae, pupae and adults along their body lengths, percent emergence and longevity of adult survival were recorded for above mentioned pests. For this purpose *S. exigua* was provided with all the four selected feeds. Larval duration was recorded to be maximum of 14.00 ± 0.47 days, and minimum of 9.00 ± 0.00 days when larvae fed on cauliflower and turnip respectively. Average larval length was recorded to be 35.4 ± 0.00 mm for cauliflower and 15.73 ± 0.46 mm for turnip. Significant difference was observed among larval growth and duration on four crops ($p < 0.05$). Average maximum pupal length of 18.33 ± 0.47 mm was attained in least duration of 9.00 ± 0.82 days on cauliflower. It took significantly longer duration ($P < 0.00$) but showed least growth on turnip (14 ± 0.82 d, $L = 15.4 \pm 0.43$ mm). Adult longevity was maximum on brassica (4.33 ± 1.25 d, $L = 23.00 \pm 0.81$ mm) and cauliflower (4.33 ± 0.47 d, $L = 26.00 \pm 0.47$ mm), followed by cabbage (4.00 ± 0.81 d, $L = 24.67 \pm 0.82$ mm), and turnip (4.00 ± 0.00 d, $L = 21.33 \pm 1.25$ mm). Value of $p < 0.01$ showed significant difference between adult longevity and growth on four crops. A total of 70% successful adult emergence was recorded for cauliflower followed by 65% for cabbage and 55% both for brassica and turnip, as mentioned before in foraging efficiency of *S. exigua*.

DIVERSITY AND RELATIVE ABUNDANCE OF BRACONID LARVAL PARASITOIDS IN CROP SYSTEM OF FAISALABAD.

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Parasitoid is a term derived from parasite, and specifically refers to insects that parasitize other insects when they are immature, but are free living when adult. They can be identified within the larger category of parasites because they eventually kill their host after completing development. Identification and conservation of these biological control agents is a basic implementation while relying least on chemicals so, as to reduce their impacts on ecosystem, especially the agro-ecosystems. A large range of braconid parasitoids attack pests like those among Lepidoptera, Coleoptera and Diptera. A total of 2533 specimens from the family Braconidae was collected and identified as belonging to 10 species of five genera. The species were *Cotesia vestalis*, *Cotesia flavipes*, *Cotesia congregata*, *Cotesia plutella*, *Heterospilus* spp. *Heterospilus eurostae*, *Bracon hebetor*, *Bracon uromelas*, *Alysia incongrua* and *Biosteres arisanus*. The fodder crop was rich with respect to the braconids. Here, a total of 808 specimens (31.90% of all crop samples) representing nine species except *A. incongrua* were sampled in this crop. The most abundant species in this crop was *C. vestalis*, represented by 29.21% specimens in the whole braconid sample, whereas, *B. uromelas* with 3.22 % was the least abundant species. Brassica harbored 21.63 % specimens belonging to six species. A total of 643 specimens (25.39 %) was collected from the vegetable crops belonging to nine species. *B. arisanus* was not found from vegetables throughout the sampling duration. *C. flavipes* (25.82 %) was the most abundant species while, *Bracon uromelas* (0.31%) shared the least portion in the vegetables. *C. flavipes* was most common (34.67 %) in the brassica crop, while *C. plutellae*, *H. eurostae*, *B. uromelas* were not present in this crop. As many as 21.08% braconids belonging to eight species were recorded from wheat. On the whole, *C. vestalis* (27.99%) and *C. flavipes* (23.53 %) were the most abundant species and were found in all the four crops. *B. arisanus* was quite rare, being found only from fodder. The diversity (Shannon diversity index, H') was significantly higher ($p < 0.05$) for fodder than all the other three crops. It was further confirmed by t-test ($p < 0.05$) and results were highly significant between fodder and other crops. The ordination of Canonical Correspondence Analysis of braconids confirmed that crop type seemed to be an important factor in the distribution of braconid larval parasitoids, especially of *C. flavipes* and *C. vestalis* which showed association with wheat, brassica and vegetables.

SPATIAL AND TEMPORAL DISTRIBUTION OF FAMILY CARABIDAE IN CEREAL CROPS OF DISTRICT FAISALABAD

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The present study was aimed to investigate spatial and temporal distribution of family Carabidae in some selected cereal crops of district Faisalabad. During an extensive survey of one year, a total of 145 samples was collected fortnightly. Sampling was done by hand picking and pitfall trap method. Sampling was carried out fortnightly from August 2014 through April 2015.

The data was subjected to Shannon-Weiner diversity index. The Shannon Weiner diversity index for three crops (maize, rice and wheat) after statistical analysis was computed as 1.57. The highest diversity index was computed in rice crops 1.41 and least was found in wheat crops 0.86. The comparison showed that there was no significant difference between the carabid abundance in the selected sites of district Faisalabad. The correlation between environmental factors and overall carabid abundance showed that the relationship between relative humidity and the overall abundance with the value of -0.884 was highly significant whereas between wind speed and overall abundance with the value of 0.669 was significant. The other two environmental factors temperature and rain fall with the values of 0.610 and 0.237 respectively had no significant impact on the overall abundance.

RESPONSE OF MAIZE STEM BORER (*CHILO PARTELLUS*) TO THE FERTILITY MANAGEMENT OF MAIZE (*ZEA MAIZE*) CROP

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The present study was conducted to check the response of maize stem borer to the soil fertility management on the maize crop; at experimental Farm, Faculty of Agriculture Rawalakot, during the year 2014-15. Maize variety Kashmir Gold was sown in May 2015 using RCBD with three replications and seven treatments. Following treatments were used: T₁, Control; T₂, Urea nitrogen 120 kg/ha; T₃, Urea nitrogen 150 kg/ha; T₄, Poultry manure equivalent to 120 kgN ha⁻¹; T₅, Poultry manure equivalent to 150 kg N ha⁻¹; T₆, Urea nitrogen 60 kg/ha + Poultry manure equivalent to 60 kg N ha⁻¹; T₇, Urea nitrogen 75 kg/ha + Poultry manure equivalent to 75 kg N ha⁻¹. Results of analysis of variance showed significant differences among all the treatment. Infestation of maize stem borer was maximum in plots where higher dose of nitrogen was applied and infestation was minimum in plots treated with higher dose of poultry manure and in control treatment. Combined application of both poultry manure and nitrogen significantly increased stem diameter, plant height (cm), ear length (cm), ear diameter, number of rows per ear, number of grains per ear and thousand grain weight (g).

SEASONAL VARIATION OF BRACONID LARVAL PARASITIDS IN CROP SYSTEM OF FAISALABAD

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A study was conducted on seasonal variation of braconid larval parasitoids from district Faisalabad. A total of 2533 specimens from the family Braconidae was collected and identified as belonging to 10 species of five genera. The species were *Cotesia vestalis*, *Cotesia flavipes*, *Cotesia congregata*, *Cotesia plutella*, *Heterospilus* spp. *Heterospilus eurostae*, *Bracon hebetor*, *Bracon uromelas*, *Alysia incongrua* and *Biosteres arisanus*. Results indicated that summer shared least 1.67 % of the total population, while maximum braconid relative abundance was

observed during spring season (62.18 %). During winter, 26.61 % of the total population was recorded and autumn shared 9.55 % of the total population sampled. *C. flavipes* was the most abundant species during summer (0.55%) and *H. eurostae* was least (0.04%), while *B. arisanus* and *Bracon euromelas* were absent during summer. A similar trend was observed for *C. flavipes* during autumn as it was then relatively abundant species, with 2.53 percentage. *B. uromelas* was the least with 0.04 percentage. During winter, all the 10 species were collected and *C. vestalis* was the most abundant species, with the 6.51 % relative abundance and *B. uromelas* was least abundant species (0.36%). Spring was the most preferred season by all the braconid species, as all of these showed maximum relative abundance. During summer the share of *C. vestalis* was maximum 18.91%, while *B. uromelas* was minimum (0.71%). Significant differences in diversity were found between summer and winter, summer and autumn and autumn and spring while non-significant results were found for summer and autumn and summer and spring. Canonical Correspondence Analysis (CCA) was applied to check the effect of different environmental factors, such as temperature, relative humidity, wind velocity and rainfall on distribution of members of family Braconidae. The canonical correspondence analysis ordination of braconids revealed that these environmental factors were important factors in determining distribution of braconid larval parasitoids in an area

SEASONAL VARIATION IN RODENT POPULATION IN CROP FIELDS AND ADJACENT NATURAL VEGETATION IN DISTRICT CHAKWAL

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The rodents are widely distributed and a serious agricultural pest in Pakistan. Relative abundance of the field rodents changes between seasons. To explore this behavior, field surveys were conducted in district Chakwal of the Pothwar area, Pakistan over a period of one year, covering all the seasonal crops and agricultural practice. Live burrow counts and trapping of rodent species was carried out at flowering, maturity, sowing and tillering stages of wheat, groundnut, millet and maize crops. Four types of food baits (tomato, potato guava, and peanut butter) on locally manufactured snap traps were used for trapping of rodents. The count of burrows density revealed that in all four crops (wheat, groundnut, millet and maize), maximum number of burrows was recorded at their maturity stages, presumably due to higher surface activity of the rodent species because of favorable climatic conditions, good shelter and plenty of food. Moreover, the study showed that the maturity stage of wheat crop coincided with spring breeding season while the maturity stages of millet/maize and groundnut crops matched with monsoon/autumn breeding peak of the rodents inhabiting agro-ecosystem of the Pothwar area. It was evident that wild vegetation like *Cynodon dactylon*, *Achyranthes aspera* and *Aerva javanica* on crop field boundaries plays an important role for providing shelter/cover to the rodent during non-crop season. The trapping data indicated that guava was the most preferred bait as compared to the rest of three baits. The outcome of this study helps to suggest that the farmers should periodically carry out rodent trapping at the beginning of each crop season and during non-breeding seasons when the populations are low in numbers and restricted under crop boundary vegetation, particularly during very hot and cold months.

PCR BASED SCREENING OF *WOLBACHIA* IN HOST PARASITOID MODEL SYSTEM, *AENASIUS ARIZONENSIS* (GIRAULT) (HYMENOPTERA, ENCYRTIDAE) AND *PHENACOCCLUS SOLENOPSIS* TINSLEY (HEMIPTERA: PSEUDOCOCCIDAE)

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Wolbachia is an endosymbiotic bacteria has a tremendous potential as biocontrol agent for the control of wide range of agricultural pest insects. It is the most prevalent and influential bacteria infecting a wide range of arthropods by inducing reproductive abnormalities like cytoplasmic incompatibility, parthenogenesis, feminization and male killing, thus affecting biology and ecology of its hosts. The presence of *Wolbachia* in host parasitoid model system *Aenasius arizonensis* (Girault) (Hymenoptera, Encyrtidae) and cotton mealy bug *Phenacoccus solenopsis* Tinsley (Hemiptera, Pseudococcidae) was studied. Practical approaches, briefly included DNA extraction from the parasitized mealybugs used in PCR reactions. A Universal eubacterial primers fD1 5'CCG AAT TCG TCG ACA ACA GAG TTT GAT CCT GGCTCAG3' and rD1 5'CCC GGG ATC CAA GCT TAA GGA GGT GAT CCA GCC3' were used for the amplification of 1492 bp region of the 16S rRNA gene and was optimized for diagnostic PCR. PCR was performed in a 50- μ L reaction with primer 16S rRNA using 1 μ L of DNA and following the profile 94.8C, 2 min; 94.8C 30 s, 58.8C 1.5 min, 72.8C 2 min (35 cycles); 72.8C 5 min. PCR products were separated on 1% agarose gel, ethidium bromide stained DNA bands were visualized under UV illumination. Insect parasitism by parasitic wasp is difficult to study, usually involving minute organisms embedded within other organisms that are themselves very small. Endoparasitism by *A. arizonensis* was detected by a multiplex PCR based technique in its host cotton mealybug *P. solenopsis*. A simple PCR assay was performed for the screening of *Wolbachia* by designing a set of primers that specifically amplify diagnostic fragments (1492 bp) of the *Wolbachia* 16S rRNA gene. *Wolbachia* infections in insect species were identified and characterized by using 16S rRNA primers as gene markers. This PCR method is more sensitive than dissection for detecting wasp DNA, even host has been parasitized less than 24 hours earlier, suggesting a potential to improve the accuracy of estimates of parasitism rates for field application of biocontrol agents. The obtained information is very helpful in devising *Wolbachia*-based biological control strategies for sustainable pest management programmes.

COMPARATIVE EFFICACY OF SYNTHETIC AND ORGANIC PESTICIDES AGAINST COCKROACHES

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Cockroaches are well known pests having 4600 species, infest wide range of environments around the world. Cockroaches cause damage in different ways as they are too ugly to live,

responsible for disease transmission, allergies and also cause feed damage. As these are mostly found in kitchen, so due to health hazard problems in using synthetic insecticides, this study was carried out. Experiment was conducted by using synthetic pesticides including boric acid and permethrin compared with organic pesticides including neem extract, bitter gourd and garlic. Rearing of cockroaches was done by providing suitable food and breeding ground. Preparation of plant extracts was done by using ethonal as solvent with soxhelt's apparatus. Different concentrations (200, 400, 600 and 800 ppm) of synthetic insecticides and plant extracts was applied by using filter paper and through spray bottles on all (egg, nymphs and adult) stages of cockroaches. Mortality was noted after 24, 48, 72 and 96 hrs. More than 80% mortality was seen in all treatments with 800 ppm concentration after 96 hrs. The LT_{50} values with 800 ppm were 28.37, 38.13, 40.64, 46.43 and 48.37 hrs with *Azadirachta indica*, *Allium sativum*, *Momordica charantia*, permethrine and boric acid respectively. The natural materials like Neem and Bitter gourd have the properties of a safe insecticide, such as proved to be effective against cockroaches, *Periplanata americana*. They are relatively biodegradable, less toxicity to non target organisms, easily available, easy accessibility, economically cheap, renewable. So plants can be considered as good substitute to synthetic and biological method of insect control.

CYCLOPHOSPHAMIDE INDUCED HISTOPATHOLOGICAL AND BIOCHEMICAL ALTERATIONS IN RAT LIVER AND PREVENTIVE ROLE OF CURCUMIN

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Cyclophosphamide is an alkylating agent used in chemotherapy, which has potential to cause organ toxicity by the generation of reactive oxygen species. Curcumin is an antioxidant substance which is used to cure many diseases. The present study was designed to evaluate the protective role of curcumin on rat liver against cyclophosphamide. 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 cyclophosphamide at the dose of 10mg/kg, i.p. The third group served as a positive control and received an oral dose of (50mg curcumin/kg bw/day). In the fourth group, curcumin (50 mg/kg) was co-administered with cyclophosphamide (10mg/kg, i.p). All the treatments were carried out for 7 days. Cyclophosphamide treatment resulted in significant histological alterations in the liver of rat and increased serum values of ALT and AST. Cyclophosphamide 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 liver tissue of rat. However, curcumin treatment significantly ($p<0.05$) recovered all the damages caused by cyclophosphamide. On the basis of these findings, it was concluded that curcumin may be used to ameliorate cyclophosphamide induced liver damage.

**IMPACT ANALYSIS OF FOOD RESOURCES, HOST AVAILABILITY AND
INSECTICIDES ON THE EFFICIENCY OF CABBAGE APHID PARASITOID
DIAERTIELLA RAPAE (M'INTOSH)**

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Biological control has three general approaches i.e., importation, augmentation and conservation. Conservation of natural enemies is a critical basic need of conservation methods under field conditions and may enhance or limit the efficiency of a particular natural enemy of herbivorous insect pests. It is assumed that extra sources of carbohydrate food could enhance the fecundity of female parasitoids. It could depend on host and food availability, mating and rate of survival of females. The experiment was performed on aphid parasitoid *Diaeretiella rapae* to evaluate the impact of host and other food resources on parasitoid's fecundity, survival and efficiency. The effect of different insecticides [Advantage® 20 EC (Carbosulfan), Imidacloprid, Polo® 500 SC (Difenthiuron) and Match® 0.50 EC (Lufenuron)] was also evaluated in the laboratory. It was observed that females fed on aphid honeydew; this diet appears to be of poor quality as compared to other sugar sources like honey and sugar solution. Second one has apparent positive impact of mating on monogenesis. The negative impact was observed on oviposition activity on female reared in laboratory as less number of mature eggs was observed in the laboratory reared females than field collected parasitoid females. All insecticides demonstrated dose dependent mortality of *D. rapae*. Conclusively, both food and host availability as well as insecticides affect the efficiency of *D. rapae*. This could lead to hypothesis that *D. rapae* females die in field after having infested only few aphid hosts.

OCCURRENCE OF INSECT PESTS OF ONION FROM DISTRICT MANSEHRA

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During the present study insect pests of Onions were determined at various localities of district Mansehra during the year 2016. Immature stages of *Atractomorpha crenulata* (Fabricius), Adults of *Chrysodeixis chalcites* (Esper) and Hymenopteran flies were recorded. Furthermore, the identification and distribution of pest species have been provided. Hopefully present study will be beneficial for pest control agencies.

EFFICACY OF IMIDACLOPRID IN COMBINATION WITH SYNTHETIC RESINS ON KAIL WOOD AGAINST *ODONTOTERMES OBESUS* (RAMBUR)

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Termites are one of the major wood deteriorating agents in the tropics and subtropics. These are well known for their capacity to damage and destroy wood and wood products. The increasing rate of deforestation necessitated to protect wood from biodegradation in order to extend its service life. The goal of current study was to investigate the wood preservative potential of imidacloprid in combination with synthetic resins. For impregnation, wooden stakes of dimension 15cm x 5cm x 2cm of Kail (*Pinus wallichiana*) were used. Imidacloprid alone and in combination with a synthetic resin was applied by surface coating and dipping methods. In addition, Wooden stakes were also oven dried before the application of chemicals at three distinctive temperatures i.e. 100, 150 and 200 °C for 48 and 72 hours to determine the effect of seasoning and chemical application against termites. Weight loss was calculated by taking weight of wooden stakes before and after four week exposure to termites in the field. In all experiments treated sap wood of kail showed more weight loss as compared to treated heart wood. Results showed that highest concentration of imidacloprid in combination with resin performed excellent as compared to other concentrations in all methods of application. Dipping of wood for 12 hours showed significant difference from other methods of application. Reducing moisture content of kail wood up to 20% with 5% imidacloprid+ resin give the best result. Drying of kail wood at 150 °C followed by application with five percent imidacloprid + resin was highly effective against the termite.

EFFICACY OF SOLIGNUM, BIO-WOOD AND BITUMIN ON THREE WOOD PRODUCTS AGAINST *ODONTOTERMES OBESUS* (RAMBUR) UNDER FIELD CONDITIONS

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The need to protect wood against biological attack with the objective of prolonging its useful services has made wood preservation an integral component of wood industry. A study was designed to protect three type of wood products i.e. chip board, moulding and veneer by using three different protecting chemicals (Solignum, Bio-wood and Bitumen) against subterranean termites. Small pieces of dimension 15 x 5 x 2 cm of each wooden material were treated with four concentrations of each chemical by dipping and brushing methods. Wooden stakes were also oven dried before the application of chemicals at three different temperatures for 48 and 72 to determine the effect of seasoning and chemical application against termites. In addition to this, a combined effect of Solignum and Bio-wood as wood preservative was also studied by mixing them in 1:1and 1:5 ratio using best concentration and best application method. Stakes of all experiments along with control treatment were exposed to termites in the field for four weeks. Weight loss of each wooden material in each experiment was calculated. Results showed that highest concentration (20%) of solignum, biowood and bitumen performed excellent as compared to other concentrations in all

methods of application. Dipping of wood for 12 hours showed significant difference from other methods of application. Temperature treatment of 200°C and moisture reduction up to 15% combined with application of chemicals was found to be more effective in protecting woods from *Odontotermes obesus*. Results also confirmed that solignum is more economical and environmentally suitable in protecting wood as compare to other two chemicals.

**EVALUATION OF MUNGBEAN GENOTYPES FOR RESISTANCE TO
BRUCHID BEETLES 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. Under Joint PSF Research Project with Mungbean Breeding Group at NIFA on "Breeding for Bruchid Resistance in Mungbean" research work was carried out with an objective to identify bruchid resistant genotypes and incorporation of resistant genes in local high yielding well adapted genotype. Culture of bruchids beetle was maintained on bold mungbean grains at 28 + 2°C and 70 + 5% relative humidity. Insect of uniform age males and females were collected separately by isolating mungbean grains in small transparent glass test tubes mouth plugged with cotton.. The stock culture maintained was utilized for conducting the experiment. Four coded mungbean genotypes were supplied by breeding group were evaluated to ascertain their resistance to bruchid beetles. The resistance of mungbean genotypes was evaluated on the basis of oviposition on grains, grain infestation and % grain damage. The results that out of four coded genotypes, three genotypes i.e. A, C and D had grain infestation of 70.25%, 55 and 55. 50% associated with grain damage of 25.75%, 44.25% and 53% respectively. Only a coded genotypes B was found with minimum grain infestation of 25% having 5% of grain damage. The conclusion is that genotype found tolerant to bruchid will be utilized in mungbean breeding program for incorporation of the desired resistant characters in the local adapted mungbean genotypes.

**EFFECT OF NON-REPELLENT INSECTICIDES AND INSECT GROWTH
REGULATORS ON THE TUNNELING AND TRAIL FOLLOWING BEHAVIOUR OF
HETEROTERMES INDICOLA (WASMANN) (ISOPTERA: RHINOTERMITIDAE)**

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The *Heterotermes indicola* (Wasmann) is economically most important and destructive subterranean termite species of Pakistan. Laboratory studies were conducted to evaluate the impact

of various concentrations of non-repellent insecticides (fipronil, indoxacarb, chlorfenapyr, imidacloprid) and insect growth regulators (hexaflumuron, lufenuron) on tunneling and trail following behaviour of *H. nicola*. Results showed that sand soaked with > 1ppm of fipronil restricted tunneling and created an effective chemical barrier against termite workers whereas < 0.5 ppm of fipronil did not affect the trail following ability of termites and 70 - 90% workers successfully completed the trail. Tunneling was greatly reduced in sand treated with > 5 ppm of indoxacarb but mortality remained < 50% whereas in 50 ppm treated sand 100% mortality was recorded. Indoxacarb at < 20 ppm did not affect trail following. The termite workers tunneled freely in sand treated with 1 ppm of chlorfenapyr and mortality recorded was up to 91.6%, indicating that chlorfenapyr could be good candidate for soil treatment. Chlorfenapyr also did not affect the trail following of termites at < 3 ppm. 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. Trail following ability was not affected significantly at all the tested concentrations of hexaflumuron and lufenuron.

**POPULATION DYNAMICS OF MUSTARD APHID *LIPAPHIS ERSYIMI* KALT.
(HOMOPTERA; APHIDIDAE) ON BRASSICA.**

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Brassica crops are important for their food and feed value. Among various aphid species attacking brassica; mustard aphid *Lipaphis erysimi* (Homoptera; Aphididae) is the serious most one. The sap feeding habits of adults and nymphs from leaves as well as stems reduces the plant vigor and result into significant yield losses. Studies were carried out at Nuclear Institute of Agriculture and Biology (NIAB) to determine the population dynamics of *L. erysimi* on brassica varieties during year 2015-16. Two varieties; Punjab Sarsoon and Faisal canola were sown at NIAB fields following Randomized Complete block design (RCBD). It was found that the aphid infestation started during last week of January, continued increasing till second week of March and declined afterwards. The highest average number of aphids per plant (shoots + leaves) were recorded in the second week of March (105.00) whereas; it was decreased (57.00) in the last week of March. Average number of aphids per plant was minimum (19.00) in the first week of April and aphid's infestation decreased towards crop maturity. Within plant distribution pattern of aphids showed that maximum number of adult and nymphs of *L. erysimi* were located in the bottom and middle parts of the plant throughout the crop growing season.

**COMPARATIVE STUDY ON PUPAL RECOVERY OF *BACTROCERA DORSALIS*
(DIPTERA: TEPHRITIDAE) ON DIFFERENT MANGO VARIETIES IN SINDH PAKISTAN**

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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 5th 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 *Bactrocera dorsalis* is tiny insect widely distributed throughout the Asia, mostly tropical and sub-tropical areas. It infested more than 250 host plant of fruit and vegetables. In Pakistan *Bactrocera dorsalis* causes serious infestation in mango, citreous and guava it causes 5-10% loss in mango. Present study was based on the sensitivity of four commercially important mango varieties Chunsa, Sindhri, Beganpali and Sonaro in Sindh to the attack by *Bactrocera dorsalis* was assessed through a series of laboratory based experiments as well as some fruit quality parameters. The developmental periods of immature stages of flies in the varieties were also determined. Pupal recovery was determined by counting and comparing the number of puparia recovered from the different varieties after exposure to the flies in cages. Chunsa was found to be the most susceptible followed by Sindhri, Beganpali and Sonaro varieties. Flies took significantly ($P < 0.05$) longer periods to complete development on the least susceptibility variety Sonara variety (25.3 ± 2.3 days) than on the most susceptibility Chunsa variety (16.8 ± 0.086).

THE PREVALENCE OF *HELICOVERPA ARMIGERA* ON THE TOMATO CROP IN DISTRICT TANDO ALLAHYAR, SINDH PAKISTAN – A CROSS SECTIONAL STUDY

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Helicoverpa armigera is a polyphagous notorious pest of a number of economic crops including tomato. *H. armigera* is a pest of major importance in most area wherever it occurs, damages a wide variety of horticultural and agricultural crops. It is widely distributed in Africa, Oceania and Asia. In Pakistan, the studies have been done but there is no work done on tomato crop in district Tandoallahyar, Sindh so the population data was taken from the district from 3rd November to 29th December 2016. Cross sectional investigation was done using the map of district. Screening of the tomato crop genotypes was conducted in the different agricultural fields of district and the *Helicoverpa armigera* was picked mechanically by hands and was saved alive/ dead in the shopper prior to the laboratory studies. Finally it was statistically counted. The results tell the prevalence of *Helicoverpa armigera* is present highly in District Tandoallahyar. Total 128 agricultural fields (average 3-5 acres per field in area) were visited in the study. The total number of 43 *H. armigera* were collected (33.5% P. rate) from 128 fields which show the high prevalence rate of *H. armigera* in the district. Further studies are continuing on its taxonomy and treatment of tomato crop.

OCCURRENCE OF INSECT PESTS OF TOMATO FROM MANSEHRA DISTRICT

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During the present study insect pests of tomatoes were determined at various localities of Mansehra district during the 2016. White fly, Aphids, Lepidopterans and leaf minors were recorded.

Furthermore, the identification and distribution of pest species have been provided. Hopefully present study will be beneficial for pest control agencies.

**RELATION OF DIAMOND BACK MOTH (*PLUTELLA XYLOSTELLA*) WITH
DIFFERENT PARASITIDS**

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Present study was carried out to investigate the relation between the diamond back moth (*Plutella xylostella* (Linnaeus, 1758)) with respect to its parasitoids. It was observed that the diamond back moth is severely attacked by parasitoids. These parasitoids play a significant role in controlling the population of diamond back moth. Hopefully, it will be beneficial for pest control agencies to control this pest.

**SYMBIOSES IN KATYDIDS AND GRASSHOPPERS NEGLECTED PARAMETER IN
PEST AND DISEASE CONTROL**

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Katydids and grasshoppers are important players in biological control as pests, control agents and transmitters of many invertebrate diseases. Katydids and Grasshoppers are repeatedly infected with one or several micro-organisms, aiding as micro-ecosystems in which multiple interactions can arise. These micro-organisms contain disease agents and symbiotic micro-organisms. The final is usually vertically transmitted and can have a wide-ranging spectrum of effects on their hosts, ranging from reproductive manipulations to protection against natural enemies. These interactions may directly or indirectly change the biology of many Katydids and grasshoppers in agriculturally, medically and ecologically important ecosystems. Symbiotic micro-organism induces reproductive manipulations such as cytoplasmic incompatibility and parthenogenesis induction can substantially affect the rearing of biological control agents. Numerous insects, lately also mites and nematodes, have been found to be infected, displaying a wide range of effects. Some of Katydids and Grasshoppers-micro-organism interactions and effects, which could have consequences for the practical application as a biological control. Symbiotic micro-organisms can also be involved in host protection against natural enemies such as parasitoids, pathogenic bacteria, fungi and viruses. Symbiotic bacteria can influence the vector capability of disease causing Katydids and Grasshoppers and may be supportive in decreasing the transmission of disease agents. The effect of micro-organisms on the product of biological control programmes is usually not considered in risk calculations and failure analyses. The present observation highlights the importance of endosymbiotic micro-organisms in comprehensive biological control programmes and offers endorsements on how to diagnose, avoid or benefit from these influential occupants.

**AGRICULTURAL PEST OF WINTER VEGETABLES IN DHAMRAH, DISTRICT
LARKANA, SINDH, PAKISTAN**

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Larkana district is an important agricultural district of Sindh because of its fertile land and variable agricultural crops and other flora. Insects are important group of animal fauna and play an important role in the biodiversity and ecosystem. Present study was carried out in Dhamrah area of Larkana district, from October to December 2016. Different agricultural crops (brinjal, tomato, spinach, chilies and cabbage and Turnip) were visited however maximum number of insect species were collected from brinjal, spinach and tomatoes. White fly, Jassid, Thrip, Aphid, Mealy bug were collected in huge number from brinjal while in Spinach Aphid, Army worm and *Helicoverpa* were found in large number. In tomatoes *Helicoverpa*, Thrips, jassid and Mealy bug were found.

**THE EFFECT OF SYNERGISTS ON SUSCEPTIBLE AND SELECTED RESISTANT
STRAINS OF *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE)**

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The combine effect of synergists (PBO, DEM and DEF) with insecticides *viz.*, trichlorfon, malathion bifenthrin, lambda-cyhalothrin, spinosad, cypermethrin and chlorpyrifos revealed that PBO showed higher synergist ratio (SR) with pyrethroids (Bifenthrin, lambda-cyhalothrin and cypermethrin) in susceptible strain as well as on three resistant strains (M₁, M₂ and SWL). It may be suggested the involvement of mixed function oxidases as resistance mechanism in resistant strains. The combination of organophosphates (Trichlorfon, malathion and chlorpyrifos) with DEF resolved as higher SR in susceptible and resistant strains. It is suggested that due to DEF with organophosphates, esterases inhibition occurred which may be responsible for decreasing of resistant ratio. The synergist ratio of different insecticides with DEM showed no significant difference among susceptible and resistant strains. It is concludes that the synergist PBO gave more toxic effect when combined with pyrethroid, while DEF+ organophosphates was successful to reduce the resistance in organophosphate resistant *B. zonata* strains.

BIOLOGICAL CONTROL OF TOMATO FRUIT WORM, *HELICOVERPA ARMIGERA* (HUB.) THROUGH EGG PARASITOID, *TRICHOGRAMMA CHILONIS* (ISHII) IN TOMATO AND OKRA CROPS IN KP PROVINCE

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Vegetables play a vital role in Pakistan's economy which contribution (10-11%) in Agriculture sector. Among borers, *Helicoverpa armigera* (Hub.) is the major economic pest of tomato and okra crops. These borers are effectively control through egg parasitoid, *Trichogramma chilonis* (Ishii) which is an important environment friendly, non-pollutant, cheaper, long lasting, and self-perpetuating and the most compatible IPM tool. Field experiment was conducted to see the parasitizing effect of *T. chilonis* against tomato fruit worm, *H. armigera*. Minimum mean incidence of fruit worm was recorded upto 1.25 borers per plant in tomato field where 800 pupae of *T. chilonis* was released followed by 600 pupae (1.52 borers/plant), 400 pupae (1.65borers/plant), 200 pupae (1.95 borers/plant) & maximum borers were found i.e., 3.87/plant in check plot (8x6 m²). All treatments means are significantly different at 5% level of probability. Tomato fruit yield results showed that the maximum mean produce of tomato was recorded in treatment 800 pupae released plot i.e. 42.17 kg per plot (8x6 m²) followed by 600 pupae (38.83 kg), 400 (36.75 kg), 200 pupae (30.83 kg) and minimum tomato produce was recorded in un-treated plot (14.83 kg) respectively. Overall results showed that treated plot with 800 pupae card of *Trichogramma* are less infested by tomato fruit worm and also tomato produce more than other treatments. Experimental results showed that the minimum incidence of okra stem fruit borers released, *H. armigera* were recorded in 800-600 pupae i.e., 0.87-0.90 borers/plant in okra plot (8x5 m²) followed by 400 pupae (1.03), 200 pupae (1.27) & maximum mean infestation was found i.e., 2.23 borers/plant in control plot where no *Trichogramma* card was installed and treatments means are not significantly different at 5% level of significance but okra treated (600-800 *Trichogramma* pupae) plots were found comparatively less infested by borers as compare to 200-400 *Trichogramma* pupal card installed plots.

OVIPOSITION AND FEEDING BEHAVIOR OF PINK BOLLWORM *PECTINOPHORA GOSSYPIELLA* LEPIDOPTERA:GELLICHIIDEA.

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Pectinophora gossypiella (pink bollworm) is also one of the major lepidopteron pests of the cotton in world and causes severe infestation. In Pakistan especially in Sindh infestation of pink bollworm increased day by day. Present study carried out in cotton growing season 2015 in field and as well laboratory. Cotton field visited fourth nightly from April to November. Adult of *Pectinophora* also reared in laboratory for oviposition on 10% sucrose solution. We have observed in the field that the females laid eggs mostly near the flowers, inside the flowers or at the base of bolls, that's why its larvae easily penetrate into the fruiting bodies and reached inside the bolls

immediately. Larvae feed on bolls mostly remain in seeds. Infested bolls not converted in cotton, the quality of cotton also not acceptable for genres. Because of oviposition behavior it is very difficult to control pink bollworm by chemical control.

**DEVELOPMENTAL PERIOD OF RED FLOUR BEETLE *TRIBOLIUM CASTANEUM*
COLEOPTERA: TENEBRIONIDAE ON STORED VARIETIES OF RICE**

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Cereals are one of the important foods for growing population of human. Approximately 50% of consumed calories by the human population depend on Wheat, Rice and Maize etc. Pakistan is one of the world's 4th largest producers of rice. Each year it produces an average of 6 million tons. The country is responsible for supplying 25% of the world's paddy rice output. Red flour beetle is one of the main grain pests of rice, both larvae and adults feed on rice grain. It makes the rice quality disagreeable and make it unfit for human consumption. Present Study carried out on two commercial varieties of stored rice Kernel basmati and Irri-09 which are cultivated at large scale in Sindh Pakistan. Adult and larval stages of red flour beetle *Tribolium castaneum* were collected from infested stored rice from market and reared in Entomology laboratory department of Zoology. To observe feeding behavior, 20 adults of red flour beetles were introduced in each variety of rice. All the development stages egg, larva, pupa, and adult of stored grains beetle were observed. During present study total seven larval instars of *Tribolium castaneum* were observed. Female starts egg laying, after 2 days of mating, egg hatched after 2-4 days, fecundity on the Kernel basmati was recorded 63% and on Irri-09 57%. The larval developmental period on Kernel basmati was recorded 34-49 days and on Irri-09, 39-50 days. Total developmental period of red flour beetle on Kernel basmati was 114-134 days and on Irri-09, 169-200 days.

**EGG PRODUCTION AND DEVELOPMENT OF *CALLOSBRUCHUS MACULATUS* (F.)
(COLEOPTERA: CHRYSOMELIDAE) REARED ON DIFFERENT STORED PULSES**

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Callosobruchus maculatus (F.) is a major pest of economically important pulses, including black eyed beans, green gram, black gram, lentils and chick pea. During present study egg production and developmental rates of *Callosobruchus maculatus* (F.) reared on cowpea, green gram, black gram and chickpea were studied. Fecundity of *C. maculatus* was high when reared on stored seeds of chickpea followed by cowpea and green gram while low on black gram. The larval periods of *C. maculatus* were longer on black gram than cowpea, green gram and chickpea. Survival and the growth response of *C. maculatus* were observed at 20-30 °C.

NEW HOST RECORD FOR ECHINOSTOMA PARAULUM (TREMATODA: ECHIMOSTOMATIDAE) COLLECTED FROM THE INTESTINE OF *CORVUS SPLENDENS* (PASSERIFORMES: CORVIDAE) IN KHAIRPUR, SINDH, PAKISTAN

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During present study on helminth parasites of *Corvus splendens*, nine trematodes of genus *Echinostoma* were collected from intestine of House crow. Specimens were pressed under slight cover glass pressure for overnight. Specimens were 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 diagnostic characteristics like body shape, number of collar spines, distribution of vitellaria, ovary and testes, the present specimens are identified as *Echinostoma paraulum* Dietz, 1909. This species is being reported from the first time from Pakistan and Common House Crow is new host record for this trematode.

STUDIES ON RODENTS ECONOMIC LOSSES IN POULTRY FARMS AND THEIR MANAGEMENT IN SUBURBS OF KARACHI

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Poultry environment is very attractive to rodents because it provides an almost unlimited supply of basic requirements that a rodent needs: harborage, food and water. Prevention of rats and mice from moving into the poultry farms is an on-going challenge. Rodents pose both qualitative and quantitative losses to poultry products by consuming poultry feed, contaminating it with their excrements, damaging eggs, attacking and killing chicks, losses to structure, nuisance value causing loss of productivity. Rodents can transmit about 45 different diseases affecting to man and other domestic animals including poultry birds. Surveys of poultry farms in the suburbs of Karachi were carried out to assess the level of infestation caused by rodents. Two species of rodents namely *Rattus rattus* (House rat) and *Rattus norvegicus* (Norway rat) were identified as dominant pest species. The population of rodents estimated through Mark-Release Capture method showed that an average 60 rats were harboring in each poultry shed. Poultry rats population was also assessed based on kill trapping for 03 nights. Trap success was computed 45.33%, Annual feed loss of 219 kg per shed worth of Rs. 1095/- was calculated. Survey of poultry farms situated in Estate II in the suburb of Karachi to determine the intensity of rodent damage to eggs in 250 sheds revealed that rats frequently damage eggs. Mean damage was calculated as 05 eggs/shed/day or total 1250 eggs/day. Annual loss of eggs was calculated as 456,250 eggs worth of Rs. 4.56 million. The success of the control trials was estimated on the basis of reduction in rodent activity. PARC bait containing Brodifacoum (0.005%) gave excellent results and 98% reduction in rodent activity was achieved.

EFFECT OF SEASONS ON TOXICITY OF SOME INSECTICIDES IN SPIDERS

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Present study was designed to assess the susceptibility of spider, *Oxyopes javanus* exposed to insecticides Emamectin Benzoate and Lambda Cyhalothrin in different seasons of the year. For toxicity test, spiders were dipped in each dose of insecticides for 10 s using a tea strainer. After 24 h spiders were categorized dead or alive by touching each specimen with fine hair brush. To check the effect of season on the susceptibility, bioassay were performed on populations of spring (March and April), summer (June and July) and autumn (October and November). In all seasons, Lambda Cyhalothrin was more toxic as compare to Emamectin Benzoate, as LC₅₀ and LC₉₀ values of Lambda cyhalothrin were well below the recommended field application rate. For both insecticides, least toxicity was observed in autumn and highest in summer season. Susceptibility of *O. javanus* also varies with the age of the specimen. Young were more susceptible to both insecticides as compared to adults in all seasons. Among adults, male were more susceptible to both insecticide as compare to female in all studied seasons. From this study, it is concluded that all the insecticides should be used in a specific quantity in the fields, so that they would not harm any beneficial organism or natural predator. The insecticides in which toxic effect show positive correlation with temperature should be used cautiously.

IDENTIFICATION AND IMPACT OF INSECTS FEEDING ON THE STORED SEEDS IN BAHAWALPUR, PUNJAB (PAKISTAN)

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Identification and impact of Insects Feeding on the stored cereal grains was investigated in Bahawalpur (Punjab, Pakistan) from August 2015 to February 2016. Stored cereal grains are presented to various insect pests that cause decrease in grain quality and add to worldwide storage losses. This study aimed to recognize insect pests that feast upon cereal grains and to find out their impact on stored seeds. The seeds of oats and maize were selected and put in containers and bags. Then these were kept in a modern store or conventional kitchen. Perceptions were made on (1) insect richness and (2) species diversity and (3) damage assessment (by evaluating the weight loss, moisture content, and germination rate of the seeds). Eighteen insect pests' species were recognized, which had a place with 15 families and 7 orders. Species richness and diversity were significantly ($P < 0.001$) higher in the kitchen and bags compared to the store and containers. In both the store and kitchen, seeds in bags had significantly higher ($P < 0.001$) humidity and weight loss rates compared to seeds in containers. In this manner, containers diminish insect pest's expansions and related damage to seeds (keeping up higher germination rates).

**DEVELOPMENTAL TIME PERIOD AND MORPHOMETRIC MEASUREMENT OF
DIFFERENT LIFE STAGES OF CIGARETTE BEETLE,
LASIODERMA SERRICORNE IN CORIANDER**

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Coriander as a spice is an important part human diet. It is rich in vitamins and minerals. Most of the spices are prone to insect infestation during storage. Coriander is included in stored spices which severely attacked by cigarette beetle, *Lasioderma serricorne*. Present study was conducted during September to October 2016 at $27\pm 2^{\circ}\text{C}$ and 65 ± 5 relative humidity to examine the biology and morphometric measurement of cigarette beetle. *L. serricorne* is extremely easy to manipulate, maintain and has a very rapid life cycle on coriander. The life cycle consisted of egg, four larval instars, pupa and adult. The eggs hatch in 5-6 days. The larval duration varied from 22-24 days. The pupal duration takes 6-7 days. The results of morphometry indicated that the length of egg was 0.40 mm varied. The length and duration of all life stages of cigarette beetle varied from egg to adult. The male and female adult's length was recorded 2-3 mm. The life cycle completes in 39-40 days in coriander.

**LARVICIDAL ACTIVITY OF FIVE INDIGENOUS PLANTS (*AZADIRACHTA INDICA*,
CITRUS SINENSIS, *EUCALYPTUS CAMALDULENSIS*, *CORIANDRUM SATIVUM*, AND
CITRUS RETICULATA) EXTRACTS AGAINST *CULEX QUINQUEFASCAITUS*
(CULICIDAE: DIPTERA)**

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Urbanization and increase in population cause many problems such as improper disposal of waste materials and temporary water catchments which is the main source of increase in mosquito population. Plant extracts have insecticidal activity and it is better way to control *Culex quinquefasciatus* through the use of botanicals. Leaf extracts from five plants (*Azadirachta indica*, *Citrus sinensis*, *Eucalyptus camaldulensis*, *Coriandrum sativum*, and *Citrus reticulata*) were extracted using soxhlet apparatus. Petroleum ether was used as a solvent. After extraction solvent was evaporated at reduced pressure using rotary evaporator and obtained residue was re-imbibed in the petroleum ether to make stock solution (10%). There were five treatments of different concentrations (400 ppm, 200 ppm, 100 ppm, 50 ppm and 25 ppm) of each plant extract with three replications. Stock solution was further diluted to make the final concentrations. Experiment was carried according to CRD design. 250 ml beaker containing 100 ml of particular concentration was used for bioassay. Fifteen larvae were released in each beaker and mortality was observed after 3, 6, 12, 24, 48, and 72 hours. The observed percentage mortality was corrected using Abbott's formula. Results indicated that the leaf extracts of *Azadirachta indica* shows best larvicidal effects with lowest LC_{50} and LC_{90} followed by *Eucalyptus camaldulensis*, *Citrus sinensis*, and *Coriandrum sativum* against larvae of *Culex quinquefasciatus*. *Eucalyptus camaldulensis* and *Citrus reticulata* showed nearly same repellent activity while lowest repellency was showed by *Coriandrum sativum*.

PHOSPHINE INDUCED BIOCHEMICAL ALTERATIONS IN CARBOHYDRATE METABOLISM AND MACROMOLECULAR CONCENTRATIONS OF A STORED GRAIN PEST *TROGODERMA GRANARIUM* OVER VARIOUS EXPOSURE PERIODS

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Toxic effect of sub lethal concentration of phosphine for various exposure periods (24-120 hrs) has been investigated on carbohydrate metabolism and macromolecular concentrations of 4th and 6th instar larvae of a stored grain pest *Trogoderma granarium* (Everts). The LC₅₀ values for 4th and 6th instar larvae of susceptible population was 7.0 and 6.4 ppm while for resistant population was 6 and 5.8 ppm, respectively. Sub lethal concentration of phosphine (LC₂₀) significantly decreased the glycogen, trehalose, lipids, RNA and DNA contents of 4th and 6th instar larvae of susceptible and resistant populations, while trehalase and amylase activities were significantly increased in 4th and 6th instar larvae of both populations throughout the exposure period with reference to control. Soluble protein contents in 4th and 6th instar larvae of both populations first increased after exposure to sub lethal concentration of phosphine but then started to decrease after 72 hrs exposure period. Free amino acid contents increased in 4th and 6th instar larvae of both populations throughout the exposure period except the 4th instar larvae of susceptible population which decreased after 72 hrs. Glucose contents in 4th and 6th instar larvae of both populations first increased then started to decrease after 72 hrs exposure except 6th instar larvae of resistant population which did not show any decline in glucose contents throughout the exposure period with reference to control. These metabolic abnormalities induced by phosphine over wide range of exposure periods provide a raw metabolic data to adopt better control strategy by monitoring phosphine exposure period for this notorious pest.

PEST STATUS OF STORED GRAIN BEETLE, *CALLOSBRUCHUS MACULATUS* ON DIFFERENT VARIETIES OF CHICKPEA IN PAKISTAN

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Chickpea belongs to family Leguminosae is the fifth most important legume crop in the world and it ranks third among the world's pulse crops after dry beans and dry peas. It contains 20% protein which fulfils the protein requirement of our daily diet. *Callosobruchus maculatus* commonly called cowpea weevil, is a destructive pest of various stored products like cowpea, chickpea, mung beans etc. The present work was done to determine the pest status of *Callosobruchus maculatus* on three varieties of chickpea namely (KP-8mm, KC-12mm and KE-9mm) during September to October 2016 at 27±2 °C and 60±5 relative humidity. In present study 10 newly emerged adults of cowpea weevil taken from already maintained culture in the laboratory, introduced in 40 grams of each three varieties of chickpea. The experiment was carried out in no choice test with single replication. The results were evaluated on the basis of eggs laid/female,

developmental period, total number of adults emerged, adult longevity of female and male and weight loss. Results revealed that the *Callosobruchus maculatus* is a pest of all three varieties of chickpea however their response varied. Taking weight loss as standard parameter, variety KP-8mm (15 gram weight loss) was highly susceptible, KC-12mm (12 gram weight loss) and KE-9mm (8 gram weight loss) moderate susceptible. Based upon the weight loss, the *C. maculatus* was declared as major pest of chickpea varieties. Moreover it was also concluded that the damaged grains did not remain fit for human as well as animal due to the bad smell create by the attack of cowpea weevil.

**SEASONAL MONITORING OF TWO DIFFERENT SPECIES OF *BACTROCERA*
THROUGH INSTALLATION OF METHYL EUGENOL TRAPS IN GUAVA ORCHARD
MIRPURKHAS, SINDH**

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Bactrocera species are pests of many crops having dynamic status and accountable for the small production and low quality of fruits in Pakistan. 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 different highest i-e surface, 1 meter, 2 meters and 3 meters all were installed at Sindh Horticulture Research Institute guava orchard, Mirpurkhas with 1512.19±15.03, 1366.34±13.48, 1595.63±16.91 and 1360.73±14.05 trap catches while *B. dorsalis* catches in methyl eugenol pheromone traps with 17.13±0.10, 16.10±0.09, 18.35±0.10 and 15.62±0.10 installed at SHRI guava orchard, Mirpurkhas at the same highest of *B. zonata*. Beside this, these two species infestation was highest when the methyl eugenol pheromone traps were installed at 2m height compare to traps installed at 1m and 3m height could not catch *B. zonata* and *B. dorsalis* more than surface installed traps.

**STUDY ON LABORATORY BIO-ASSAY OF DIFFERENT PESTICIDES AGAINST
OVER-WINTERING LARVAE OF CODLING MOTH *CYDIA*
(*LASPEYRESIA*) *POMONELLA* L.**

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The prime importance of spraying pesticides on apple crops in Balochistan is to control larval infestation of codling moth *Cydia* (*L.*) *pomonella*. The evaluation of pesticides and assessment of damage by the moth larvae is more important both from agricultural and economic point of view. To reduce loss of apple fruit in order to uphold primary source of apple farmers' income, the present study was conducted. The experiments were carried out on different concentrations of active ingredients of four pesticides i.e. Fury 18.1 EC (Zeta-Cypermethrin), Lorsban 40 EC (Chlorpyrifos), Talstar 10 EC, (Biphenthrin), and Match 50 EC (Lifefenuran) to

evaluate the potency and effective doze of each chemical against over-wintering larvae collected from apple orchard trees. The Lorsban 40 EC found highly toxic (ED50 = -3.406) to over-wintering larvae of moth than Fury 18.1 EC (-4.046), Talstar 10 EC (1.721), and Match 50 EC (1.728) tested after 24 hours. Similarly, Fury 18.1 EC was observed toxic (ED50 = -1.849) against larvae compared with other insecticides after 72 hours. While Lorsban 40 EC was highly toxic examined after one week time than other three insecticides in laboratory.

**ASSESSMENT ON TRAPPING EFFICIENCY OF THREE PLANT
INFUSIONS IN OVITRAPS FOR *Aedes* VECTORS OF DENGUE IN
KHYBER PUKHTON KHWA (KP), PAKISTAN**

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The present work summarizes the results of three different plants infusions in ovitraps prepared from hay, eucalyptus and grass. Plants infusions were prepared in plastic tubs containing crushed botanical materials with tap water and allowed to ferment for 3 days. Ovitrap were prepared by using 500 ml of infusion in 500 ml tap water. All traps including control in simple water were installed in open areas near houses for 24 days at four different locations Peshawar, Swat, and Thimergera in Khyber Pukhton Khwa (KP) province. Assessment on the oviposition response of gravid female *Aedes* mosquitoes was done on the number of eggs/ trap twice a week in each ovitrap. The results showed 1876 cumulative eggs per trap from hay infusion at Peshawar followed by grass (574), eucalyptus (56) and control (42) respectively after 24 days of exposure. At Thimergera, the same pattern was noted for hay with 1958 eggs/trap followed by grass with 1808 eggs per trap and eucalyptus with 1660 eggs/ trap. At Swat more eggs were recorded from grass infusion (1666) followed by eucalyptus (1651) and hay 1616 and finally control. However, there was no significant difference between the numbers of eggs recorded from infusion traps at this site. Data on relative abundance of *Aedes* species showed both *Aedes aegypti* and *Aedes albopictus* were present in Swat with dominant number of *Ae. aegypti* while *Aedes albopictus* was dominant species at Thimergera. However, only *Ae. albopictus* was recorded from Peshawar, Overall results indicated that hay infusion-baited ovitraps are useful tools for both *Ae. aegypti* and *Ae. albopictus* surveillance and should be used in future surveillance program for *Aedes*.

**EFFECT OF GAMMA RADIATION AND COLD STORAGE ON *SITOTROGA*
CEREALELLA AND *TRICHOGRAMMA CHILONIS***

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Experiments were performed to assess the optimum low temperature and radiation dose that can enhance the existing production capabilities of *Trichogramma chilonis* and *Sitotroga cerealella*. Fresh pupae (50) of *Trichogramma chilonis* and eggs (50) of *Sitotroga cerealella* pasted on cards were radiated (doses: 35, 55, 75, 95 Gy) and stored at temperatures of (4 & 8°C) for different time intervals of (3, 5, 7, 10, 15 days), replicated three times in CR design. Results

revealed that low doses of gamma radiation and cold storage increase shelf life of *S. cerealella* and *T. chilonis* without maximum detrimental effects on the quality of parasitoid and host. It was observed that 35 Gy dose exhibited promising results with maximum hatching of *S. cerealella* and maximum adult emergence and percent parasitization in *T. chilonis* and increased the incubation period of *Sitotroga* eggs from 3 to 7 days. It was evident that, storage at 4°C was very conducive for *S. cerealella* eggs whereas, 8°C was favorable for *T. chilonis* pupae to get short term storage. These findings can ensure the year around availability of parasitoids in insectaries for research and field releases.

SOIL TYPE LIMITS, DISTRIBUTION OF RODENTS: A CASE STUDY OF SOME MURIDS, IN NORTH PUNJAB, PAKISTAN

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The present study was conducted to find rodent distribution in different soil types in North Punjab. The survey was conducted from February to December, 2016 using snap traps by applying line transect technique in wild area. A total of 1663 animals belonging to 6 species were captured in 3840 trap nights in eight different soil type (Sand, loamy sand, sandy loam, sandy clay loam, loam, clay loam, silt loam silty clay loam). The maximum number of rodents was captured in loamy soil with population density 0.33 per hectare, capture per night 0.7 and trapping success 69.8%. The lowest rodent population density was captured in sandy loam with density 0.16 per hectare, capture rate 0.3 and capturing success 33.3%. The population density in soil types, sand, Loamy Sand, sandy clay loam, silty clay loam and clay loam did not differ significantly ($P \leq 0.05$). The results of this study suggest that trapped rodents (*Mus musculus*, *Mus booduga*, *Tetra indica*, *N.indica*, *Golunda ellioti*, *Millarda meltada*) prefers loam-textured soils with percentage of sand 40%, silt 35% and clay 25%.

ISOLATION AND MOLECULAR CHARACTERIZATION OF *cyt* POSITIVE *BACILLUS THURINGIENSIS* ISOLATES AND THEIR MOSQUITOCIDAL ACTIVITY

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Vector borne diseases are becoming havoc these days including Congo and Dangué fever, and vectors of such diseases becoming more powerful and resistant to commercial insecticides day by day. The commercial insecticides have many side effects as being not host specific and also responsible for the killing of useful insects, not easily degradable and being biomagnified in natural environment. Need of the hour is to look for control agents which are host specific and environment friendly. One of such control agent is *Bacillus thuringiensis* commonly called *B.t. B.t.* produces parasporal inclusion bodies corresponding to two different types of proteins called *cry* and *cyt* proteins. Present study was focused to determine the insecticidal activity of *cyt* positive strains of *B.t.* Bacteria were isolated from different soil samples rich in organic manure. These

bacteria were subjected to biochemical characterization. After biochemical characterization the bacteria which were expected to be *Bacillus* like were confirmed by 16SrRNA gene amplification using specific primers for *B.t.* The *B.t.* isolates were then screened for the presence of *cyt* gene through PCR amplification. To check the biotoxicity of *cyt* positive strains of *B.t.* larval bioassays were performed with 3rd instar larvae of mosquito and different doses were made of *B.t.* spores and total cell proteins. GCU *B.t.* 4 is found to be most toxic against mosquito larvae followed by GCU *B.t.* 1, GCU *B.t.* 2, GCU *B.t.* 5, GCU *B.t.* 3 and GCU *B.t.* 6.

**EFFECT OF EMAMECTIN BENZOATE ON BEHAVIOUR OF *OXYOPES JAVANUS*
(ARANEAE: OXYOPIDAE)**

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Present study was designed to investigate the effect of Emamectin benzoate on locomotion, exploratory and feeding behavior of *Oxyopes javanus*. For this purpose, specimens of *O. javanus* were collected from the unsprayed field of University of the Punjab, Lahore, and exposed to the Emamectin Benzoate at the recommended field rate by dip method. In climbing test, the velocity of untreated male and young spiders was more than treated ones. However, treated females had more climbing speed than untreated females. During the climbing, inactive duration was more in treated young and male spiders but less in treated females. In open field tests, data showed significant variation between activities of treated and untreated spiders. The exploring activity of untreated spiders was more in the edge sectors while untreated spiders explored more in intermediate sectors. However, no significant difference in exploration of central sector was recorded in treated and untreated spiders. During the experiment, untreated group of spiders consumed more prey than treated group. From this study, it was concluded that insecticides cause a marked difference in the behavior of spiders which ultimately affect their biological control potential.

**MANAGEMENT OF THE TOMATO FRUIT BORER (*HELICOVERPA ARMIGERA*)
USING AQUEOUS SOLUTIONS OF BOTANICAL EXTRACTS AND
BIOCHEMICAL INSECTICIDE**

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The present research was conducted at the Agriculture Research Station, Baffa (Mansehra) during the normal cropping season, 2015. The experiment was laid out in randomized complete block design (RCBD) with application of seed extract of Neem (*Azadirachta indica*) with 2%, 5% and 7% concentrations, as well as Puluck (*Persicaria hydropiper*) at 1% and 3% concentrations against *Helicoverpa armigera*, tomato fruit borer. In first week time span (24hrs, 48hrs, 72hrs and 7days) Neem 7% showed highest efficacy. Similarly, in all 8 weeks, Neem 7% followed by Neem 5% showed significant high and moderate efficiency against *H. armigera* (0.1 and 0.18 mean number of larva) in tomato plant. Weed extract showed to be slightly effective as compare to control tomato

plant. It was concluded that Neem 7% being highly effective than local available insecticide in the market can be recommended to tomato farmers. Also further study is required for isolation of those compounds that enhance resistance in tomato against *H. armigera* as well as other tomato pathogens.

STUDIES CATEGORIES OF RESISTANCE IN COMMERCIAL CANOLA CULTIVARS AGAINST APHID (*L. ERYSIMI* K.) THROUGH PHENOTYPIC CHARACTERS

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The objective of the study was to develop insect pest management strategy by exploring host plant resistance in canola, *Brassica napus* L, ten canola cultivars viz, (Rainbow, Omega, NARC, PARC, Shira lee, Abaseen, Hoyla-401, Oscar and Zahoor) were tested for their resistance/tolerance against aphid (*L. erysimi*) in randomized complete block design (RCBD) at the new developmental farm of Khyber Pakhtunkhwa agricultural university Peshawar, during the Rabi season of 2015-16. The results of overall mean revealed that minimum aphid population on peak period (162.0/plant) was recorded on canola cultivar NARC and maximum aphid population on peak period (367.7/plant) was recorded on Zahoor. NARC resulted in produced no. of branches/plant (31.26), no. of silique/plant (4072.6), length of silique (7.09) seed/silique (26.92), plant height (132.3), plant maturation (145.0), 1000-seed /weight (14.17), while Zahoor (19.25), (2594), (5.46), (13.04), (108.3), (159.9) and (10.03). It was concluded from the study that aphid population on all the tested cultivars fluctuated and none of the cultivar showed complete resistance to aphid during most of theseason. This study will provide an opportunity to fact the pest challenge by manipulatingthe manageable ecological parameters in the form of planting or harvesting time adjustment, varieties selection, correct timeofpesticideapplication, etc. The naturalappearance of mustard aphid on variety and germplasm of mustard was observed onJanuary 06, 2015-16 (50 days after sowing) and disappeared after end of March (130 days after sowing). However, highest population was recorded during last week of February to second week of March. The peak aphid population was found at a minimum, maximum and average temperature of 16.89, 27.67 and 21.34 respectively and a mean relative humidity of 78.91% on February 27 2015-16 (102 days after sowing). Then, decline in aphid population. Correlation coefficient (r) showed a positive effect with minimum, maximum and average temperature. Whereas mean relative humidity showed significantly negative effect. However, the data on yield and other agronomical characteristics indicated that the cultivar NARC possess some levels of resistance against aphids attack at new developmental farm in 2015-16.

FORMULATION AND APPLICATION OF ENTOMOPATHOGENIC FUNGI *ASPERGILLUS* FOR PEST CONTROL

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Indiscriminate use of various pesticides is rising as a big challenge for agriculture sector. High and frequent spray adversely affects living creature and their environmental survival-ship.

Different studies were carried out to determine the effects of these pesticides but nothing was introduced as its substantial supplement. At the present, *Aspergillus* was trailed as bio-pesticide on different development stages of Acrididae species under laboratory conditions. Formulation of *Aspergillus* was prepared in water and oil based medium. Fair numbers of Acrididae species were captured from different ecological zone of Sindh during the year 2015-2016. The infected isolation percentage of entomopathogenic fungi and their association with pest species of grasshopper have been presented in comparative manner. Total No. of isolated percentage of *Aspergillus niger* was reported 6.77% and 5.64% on *Acrida exaltata* and *A. gigantea* respectively, in sub-family Acridinae, 6.21% in *Acrotylus humbertianus* of Oedipodinae and 5.08% *Truxalis eximia eximia* contaminated with *A. fumigatus* and *Oxya fuscovittata* infected 5.08% with *A. flavus*. Beside this, observations were also taken under Scanning Electron Microscope (SEM) in order to determine the pathogenicity of three *Aspergillus* species through elements counting percentage. Critical analysis of experimental trails illustrated that most relevant formulation is oil based it is more affected for longer period of time rather than water based medium. Significant mortality of insect was observed in 48 hrs after the treatment of *Aspergillus*. This bio-pesticide will be capable to protect high yield if applicable in field and ultimately will be helpful to improve socio-economic problems of farming community.

**EFFICACY OF DIFFERENT MANAGEMENT PRACTICES AGAINST CLCUV DISEASE
IN COTTON CROP BY ADOPTING FARMER FIELD SCHOOL APPROACH**

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Cotton leaf curl disease (CLCuD) is a debilitating disease of cotton crop across the cotton growing areas of Pakistan. In an effort to build up the capacity of farmers in managing CLCuV through Farmer Field School (FFS) participatory approach during 2012-16 under ICARDA-USDA project, farmers of Bahawalpur, Khanewal, and Vehari districts observed the influence of different sowing dates, treatment of seed with bio-fertilizer, urea (2%) along with micronutrients (Zinc, Manganese and Boron) and various bio-pesticides on cotton crop of minimum one acre area during agro-ecosystem analysis. It was found that biofertilizer treated seed showed better germination while 40-74% cotton plants up to disease severity scale 2 showed recovery from the virus. Less disease incidence was observed in late April (early sowing) and May (mid sowing) planting especially within two months period as compared to June sowing. The said management practice was found successful in more than 40 FFS of three districts during three consecutive cotton growing seasons. It was also found that CLCuV attack was severe in areas with uncertified crop varieties, unawareness among farmers and multi-crop fields. High incidence of CLCuV was observed in the cotton fields having wind barriers and surrounded by other crops especially Mango and Citrus orchards.

**SPECIES COMPOSITION AND ALTITUDINAL DISTRIBUTION OF FRUIT FLIES
(TEPHRITIDAE: DIPTERA) FROM POONCH DIVISION OF
AZAD KASHMIR, PAKISTAN**

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Fruit flies (Diptera: Tephritidae) are economically most important pests of fruits and vegetables worldwide. The present study lime light the identification of five species of fruit fly species viz., *Bactrocera zonata* *Bactrocera dorsalis*, *B. cucurbitae*, *Bactrocera scutellaris* and *Dacus sphaeroidalis* from Poonch division of AJ&K. The altitudinal distribution of *Bactrocera zonata* was recorded from 3500 ft to 7500ft while *Bactrocera scutellaris* and *Dacus sphaeroidalis* was found below 4000 ft from sea level. *Bactrocera zonata* was found most abundant from study site.

**PHYTOCHEMICAL ANALYSIS AND ENTOMOCIDAL IMPACT OF SOME WEED
PLANT EXTRACTS IN *CULEX* MOSQUITOES**

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Control of the mosquito and other insect pest is frequently dependent on continued applications of synthetic insecticides. But this has not been very successful due to high cost, various environmental and health concerns, their non biodegradable nature and increasing insecticide resistance on a global scale. Hence, the replacement of synthetic insecticide with a plant and microbe based biopesticide is considered successful and environmental friendly to manage insect pests. In the present study, the entomocidal impact of biopesticide and also the genotoxic effect was evaluated in *Culex* mosquitoes from Faisalabad. The collected populations from Govt. College University Faisalabad were reared separately in the laboratory. Petroleum ether extract from five different weed plants' viz. *Convulvulus arvensis*, *Chenopodium murale*, *Tribulus terrestris*, *Trianthema portulacastrum*, *Achyrrathes aspera* singly and/or in combination with *Bti* and *Psuedomonas* were used. Percentage mortalities of *Culex* mosquito were calculated and data was subjected for statistical analysis. LC₅₀ of all the tested biopesticides was determined using Probit analysis. *Achyrrathes aspera* showed 100% mortality at 250ppm, moreover, it was found that increased concentration caused increased mortality. Interestingly, the combination of *Convulvulus arvensis* and *Trianthema portulacastrum* exhibited the 91.66% mortality of *Culex* larvae. However, the combination of *Achyrrathes aspera* with *Bti* showed the least LC₅₀ value. In order to estimate the level of genotoxicity, highly significant plant extract was selected further. The DNA amplified through RAPD-PCR and DNA damage through comet assay showed no change in DNA profile compared to control. FTIR analysis showed the presence of phenolic compounds in *Achyrrathes aspera*. Based on the current results, an environmental friendly and economical biopesticide can be suggested to use for the management of *Culex* mosquitoes.

**STUDIES ON EGG DEVELOPMENT AND HATCHING OF *POEKILO CERUS PICTUS*
(PYRGOMORPHIDAE: ACRODOIDEA: ORTHOPTERA) UNDER
LABORATORY CONDITIONS**

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Poeciloceris pictus is mostly associated with Akk plants and cause considerable damage to this and surrounding vegetation. Some observations have been made on egg development and hatching of *Poeciloceris pictus* Fab under laboratory conditions. For the development of eggs of *p.pictus* maximum numbers of eggs were kept in petri dishes in different date during summer and autumn season under normal laboratory conditions where they were major hatching occurred within few weeks i.e 27.5% followed by 16.85% in month of November and minimum hatching percentage i.e 5.27% were obtained for month of September 2013 for those insects which were obtain in summer season. Beside this, very low percentage of hatching was obtained for eggs were maintained in autumn season i.e between 7.3% to 2.03% in the month of May.

SECTION – III**ENTOMOLOGY****PRELIMINARY STUDIES ON THE TRIBE SPHINGONOTINI (ACRIDIDAE:
ORTHOPTERA) FROM PAKISTAN**

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The genus *Sphingonotus* Fieber; were considered as agricultural pest. During present study Twelve species and subspecies of the tribe were collected from different localities of Pakistan as well as *Sphingonotus savignyi* Saussure, *S.hussaini* Baloch & Wagan, *S.longipennis* Saussure, *S.nebulosis tokhai* n.sp, *S.balteatus himalayanus* Uvarov, *S. balteatus balucha* Uvarov, *S. sindhensis* n.sp, *S. akbari* Wagan & Baloch, *S. maculatues petraeus* Bei-Bienko, *S.rubescens afghanicus* Mistchenko, *S.rubescens subfasciatus* Mistchenko, and *S.rubescens rubescens* (Walker). These have been described with four illustration of Phallic complex and description also provided. The significance of Orthopteroids has been taken out previously by many Scientists. Presently the distribution of *Sphingonotus* grasshoppers and key characters of the genitalia Such as Epiphallus with moderately wide bridge, narrow ancorae and bilobate lophi. Spermatheca is a coiled duct of ectodermal origin. It is of varying sizes and shapes. The spermatheca usually dilates to form a sac like structure, for storing the sperms which enter during copulation. On other hand the present study will make the unique support for new research workers in future.

**BIODIVERSITY AND BIOGEOGRAPHY OF MANTODEA: DICTYOPTERA
(PRAYING MANTIDS) IN PUNJAB, PAKISTAN**

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Biodiversity means life diversity of a particular place, habitation or when combined with biogeography becomes the study of biological diversity of environment isolated by barriers and both pronounce how and why distribution of plants and animals occurs. For this, study was undertaken to prepare a comprehensive and updated record of Praying Mantids (Mantodea) belonging to families, Eremiaphilidae, Empusidae, Mantidae Amorphoscelididae and Hymenopodidae occurring in Punjab Province of Pakistan. 296 individuals were collected and sorted into 23 species of mantids. Biodiversity, biogeography and Species richness of praying mantids calculated while using the Shannan Index H, Simpson's Index D, Simpson's Index of Diversity 1-D and Simpson's Reciprocal Index 1/D.

TAXONOMY OF SPIDERS (ARANEAE: ARACHNIDA) OF RICE FIELD IN DISTRICT DADU, SINDH, PAKISTAN

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Pakistan is an agricultural country and enjoys massive consequence in economic zone of the world. It blessed four weathers, in which various crops are cultivated including rice. Rice crop contribute more than 2.3 million tones every year. It has several insect pests like stem borer etc which causes 25-30 percent losses. Beside the other pests a predator namely spider also resides on rice which is the best alternative to the chemical control. So the present study, of rice fields was undertaken and 4 taulkas of district Dadu Sindh were surveyed and total 621 individual specimens were collected. Specimens were sorted out into five families: Araneidae, Lycosidae, Sparassidae, Tetragnathidae and Oxyopidae, six genera and eight species. The identification was done on the basis of external morphological characters with the help of keys and other relevant literature. Pictures were captured by digital camera.

THE COMPARATIVE STUDY OF EVALUATION OF NUMBER OF DAYS PER MOLT OF THREE *PARDOSA* SPIDER SPECIES

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The days per molt of *Pardosa* species viz., *P. birmanica*, *P. leucopalpis* and *P. oakleyi* was observed under controlled conditions. The experiment was performed in the Arachnology laboratory Department of Zoology, University of Management sciences and Information Technology Kotli. The spiders were nurtured on artificial diet in clear perplex cages. The ingredients of the diet were of 100ml soya bean oil, one fresh chicken egg yolk and 5ml honey. The cotton swab was saturated in artificial diet and kept in the cage. The diet was replaced after regular intervals (24 hours). The laboratory conditions were being maintained at 27±2 °C, 70±5% RH and 12:12 L: D photoperiod. After mating ten female spiders were selected for research in separate cages. Spider hatchlings cling to the female abdomen for 4 days after that they were separated from the female abdomen. The seventy spider lings were randomly selected. And each spider ling was introduced into a separate cage to prevent cannibalism. The manmade diet was given to these spider lings under same controlled conditions. Daily observation was noted on each cage to study the

developmental stages of *Pardosa* species viz., *P. birmanica*, *P. leucopalpis* and *P. oakleyi*. The first molting took place inside the egg sac. Three selected *Pardosa* species viz., *P. birmanica*, *P. leucopalpis* and *P. oakleyi* exhibited eight post-emergence molts to become adult.

LIFE HISTORY STATISTICS OF *ACROTYLUS HUBERTIANUS* ON DIFFERENT FOOD PLANTS UNDER LABORATORY CONDITION

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Acrotylus humberianus Saussure is a multivoltine and widely distributed grasshopper in Pakistan. It is voracious feeder of many economically important crops such as maize, sugarcane, sorghum, wheat, many fodder crops and wild vegetation. It's breeding capability, growth rate and survivability was very high. It was noticed that this pest undergoes in six molts and then become adult. The female grasshopper deposit eggs in mid-summer, and they remain 1 or 2 inches under the sand or in leaf litter. Each egg pod contains 15 to 150 eggs, depends on mode of energetic feeding of insect. Mostly grasshopper can lay up to 3-4 pods during entire life. The eggs remain underneath for about 10 months in autumn and winter before hatching into nymphs during spring or in the initial days of summer. Wingless nymphs consist on 5/6 stages separated by moults after each nymphal instar. Whole the nymphal development takes place in five to six weeks for conversion into adult stage. It takes about 30 days for fully development. The mature grasshopper is more mobile and active than the nymph due to development of wings that helps them to hunt and fly far away.

MORTALITY OF ACRIDID SPECIES CONTAMINATED WITH *ASPERGILLUS* UNDER LABORATORY CONDITION

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Aspergillus species are known as good bio-pesticides due to their quick ability to kill target organism in field this practice is more common in abroad but in Pakistan less attention was paid towards this. Therefore, present attempt is made. During the present study three species of *Aspergillus* i.e *A. flavus*, *A. fumigatus* and *A. niger*, excluding control were treated with Acridid species under laboratory condition. It was observed that infestation of *Aspergillus* cause significant reduction in the feeding of insect. However, mortality of insects on day first was noted significant highest i.e [F_{0.48} = 84.65, P < 0.05] followed by [F_{0.35} = 61.96, P < 0.05] and [F_{0.27} = 48.00, P < 0.05] on day 4th and 2nd respectively. It was extremely low i.e [F_{0.17} = 30.54, P < 0.05] on day 3rd. However, mortality of Acridid (nymphs) population kept in large cage when treated with conidial concentration formed in H₂O was maximum on day 6th i.e [F_{0.82} = 43.99, P < 0.05] and it was non-significant i.e [F_{8.5} = 14.84, P < 0.05] and [F_{7.25} = 13.09, P < 0.05] on 2nd and 3rd day respectively, while it was significant low on 5th day i.e [F_{3.32} = 06.11, P < 0.05], whereas, mortality of Acridid adult suggests that maximum mortality was observed on day 7th i.e [F_{13.7} = 23.56, P < 0.05]

followed by [$F_{12.5} = 21.82$, $P < 0.05$] on 6th day and minimum mortality i.e [$F_{0.44} = 77.67$, $P < 0.05$] was noted on day 1st followed by [$F_{0.77} = 35.26$, $P < 0.05$] on 3rd day. Similarly, a mortality ratio for 4th and 5th day was non-significant. The result of present study will be useful to implement the IPM techniques in future.

OVIPOSITION PREFERENCES OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA* (FORSKAL) (CYRTACANTHACRIDINAE: ACRIDIDAE: ORTHOPTERA)

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Desert locust is destructive pest of any kind of vegetation throughout world including Pakistan. They are propagating and consuming vegetation in various countries during different breeding seasons such as in Pakistan they are thriving in Baluchistan during winter breeding season, while spreading in Thar Desert Sindh during summer breeding season. In present study field surveys at various localities within desert locust breeding habitats from all over the Thar Desert was carried out to conduct field observations on oviposition preferences. This study indicated that early in the rainy season the solitary females oviposited predominantly in the vicinity of pearl millet (*Pennisetum glaucum*) and guar or cluster bean (*Cyamopsis tetragonoloba*) crops in cultivated fields, while in uncultivated land preferred the surroundings of bekar grass (*Indigofera caerulea*) and snow bush (*Aerva javanica*). Solitary nymphs also preferred to feed on these plants. Females preferred loose soil for oviposition rather than packed or hard soil and oviposited with the depth measured up to 8.996 ± 1.40 cm. During this study we also collected female from field and fetched to laboratory for rearing and we observed that gravid females oviposited on the bottom of perforated plastic cages when, they did not find suitable soil for oviposition. The data regarding the oviposition sites will be useful to detect the eggs and their destroying will be possible at early stage.

SEASONAL DISTRIBUTION OF *HIEROGLYPHUS ORYZIVORUS* (HEMIACRIDINAE: ACRIDIDAE) IN RICE ECOSYSTEM

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The *Hieroglyphus oryzivorus* (Carl) is reported as a major pest of rice, sugarcane, wheat, maize and as a minor pest of millets and fodder crops in Sindh Pakistan. During the present investigation, a total of 1709 specimens were collected from Buxapur and Badani a major rice growing areas of Kashmore. Both macropterous and brachypterous forms of *H. oryzivorus* were collected from 02 importance varieties of rice i-e Aery (Sindhi) and Hybrid (Dhaga) which are more preferable by this pest. Multiple generation of Dhagha provides the ideal breeding place for enhancement of this pest population. Beside this, its reproductive activities were also studied in controlled condition. Further, *H. oryzivorus* was also compared with *H. banian* its very closely relative member in order to highlight their major morphological differences. The results of such

study will be instrumental in understanding and devising the population management strategies to adopt control measures at early incidence time in field.

**FIELD OBSERVATION ON THE IMMATURE STGES OF OCHRILLIDA
(GOMPHOCERINAE: ORTHOPTERA)**

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Extensive field surveys were conducted in order to collect the maximum numbers of immature stages of ochrillida during the year 2015-2016. Present a total of 438 specimens were captured and sorted out into two species i.e. *Ochrilidia geniculata* (Bolivar, 1913) and *Ochrilidia gracilis* (Krauss, 1902). The specimens were brought to laboratory and were reared under the laboratory condition. It was noted that both the reared species passes through the five nymphal instars. These both are significant differ with each other beside this, morphometry difference and general characters also make them different. Knowledge on the immature will be guideline for future prediction so that proper control planning should be done at time.

**A NEW SPECIES OF GENUS GRYLLOMORPHA
(GRYLLIDA: ORTHOPTERA) FROM THAR DESERT**

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As a result of this work, a large number of specimens were captured from Diplo. Mostly the specimens were collected from agriculture land, desert and semi desert areas, include shrubs and various grasses. The collected material was sorted out into to single sub-family Gryllmorphinae. This subfamily pertaining into one tribe Gryllomorphini and single genus *Gryllomorpha* and a new species *Gryllomorpha deserti* has been described from Thar for the first time. It is distinguishing from other species in account of the following characteristics: basic coloration of the body is pale brown, with dark-brown spots, antennae very long, completely apterous body while these characters are not present in already existing species of this genus.

ON THE IDENTITY OF TETRIGIDAE (ORTHOPTERA) FROM SUKKUR

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The grasshoppers belonging to the Tetrigidae are the severe pest of the agricultural crops in the Sindh. They are the major pest of the agricultural crops, wheat, rice, maize and deserts vegetation. Pygmy grasshoppers are less than 20 mm in length, and are recognizable by a long pronotum, that extends over the length of the abdomen. These pygmy grasshoppers usually found near water, such as ponds, and streams. Occasionally found in dry habitats, woodlands, old fields, rice fields and sandy areas with lichen. These grasshoppers especially eat roots of plants, seedlings, mosses, fungi, algae and organic muck and cause considerable damage to crops. During the present study six species i.e. *Hedotettix gracilis*, *H. lineifera* (Haan, 1843) *Ergatettix dorsifera* (Walker 1871) *Paratettix femoralis* (Sjostedt 1934) *P. variabilis* (Bolivar, 1887) *P. nigrescens* (Sjostedt, 1921) were reported from Sukkur division.

RICHNESS OF ORTHOPTERAN SPECIES FROM THAR DESERT

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That desert is very peculiar amongst the other three desert of Pakistan. It has maximum number of hidden wealth of insects. But due to harsh condition it was not survey before this. Present attempt has been made for the first time. In result of extensive survey a total of 734 specimens were collected during 2014-2016 and sorted out into 3 Families i.e. Acrididae, Pyrgomorphidae and Dericorythidae, 7 Subfamilies i.e. Oedipodinae, Pyrgomorphinae, Eyprepocnemidinae, Acridinae, Tropidopolinae, Dericorythinae and Cyrtacanthacridinae, 12 Tribes, 15 Genera and 18 Species viz. *Hilethera aeolopoides* (Uvarov, 1922), *Chrotogonus (Chrotogonus) homalodemus homalodemus* (Blanchard, 1836), *Aiolopus thalassinus thalassinus* (Fabricius, 1781), *Heteracris littoralis* (Rambur, 1838), *Poeciloceris pictus* (Fabricius, 1775), *Acrida exaltata* (Walker, 1859), *Atractomorpha acutipennis blanchardi* Bolívar, 1905, *Pyrgomorpha (Pyrgomorpha) bispinosa bispinosa* Walker, 1870, *Pyrgomorpha (Pyrgomorpha) bispinosa deserti* Bey-Bienko & Mishchenko, 1951, *Tenuitarsus orientalis* Kevan, 1959, *Chrotogonus (Chrotogonus) trachypterus trachypterus* (Blanchard, 1836), *Tropidopola longicornis* (Fieber, 1853), *Dericorys tibialis* (Pallas, 1773), *Anacridium aegyptium* (Linnaeus, 1764), *Sphingonotus (Sphingonotus) rubescens rubescens* (Walker, 1870), *Sphingonotus (Sphingonotus) savignyi* Saussure, 1884, *Trilophidia annulata* (Thunberg, 1815) and *Eyprepocnemis alacris alacris* (Serville, 1838), were captured and identified from this dry land. Addition to this, identification keys were also constructed for easy recognition of taxa. Collection of huge material from this region is significant addition in wealth of Thar Desert.

REGIONAL RECORDS OF *MANTIS* (MANTIDAE: DICTYOPTERA) FROM SINDH

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New regional records of genus *Archimantis* are presented from Sanghar. This genus consists of 09 species across the world wide. In this study confirmed that presence of genus *Archimantis* with two species i.e. *Archimantis monstrosa* (Wood-Mason, 1878) and *Archimantis latistyla* (Serville, 1839). During the present study female specimens of *A. monstrosa* and *A. latistyla* were collected from khipro between range of (26°2'39.91" North latitude and 68°57'13.96" and East longitude). The appearance of these species is very unique from other mantids species. It is elongated and callous, brachypterous and oval in shape. *A. monstrosa* and *A. latistyla* morphological closely related and utilize equivalent habitats. Addition of these records enhance the wealth of this fauna from Sindh.

TEMPORAL DISTRIBUTION OF SOME LEPIDOPTERAN SPECIES FROM CROP SYSTEM OF FAISALABAD.

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Many lepidopterans are pests of economic importance. Work has been carried out for their diversity and abundance from agro-forest area of this region. Research also has been carried out for arthropod cropland biodiversity from Punjab, but no specific work has been carried out for assessing the diversity and abundance of these pests from the cropland of Faisalabad. Without determining the diversity and abundance of pest species, no methods can be used effectively. Therefore, the present study was carried out to provide some basic information about temporal distribution of some important lepidopteran pests of the cropland of Faisalabad. Sampling was carried out fortnightly from selected places of district randomly. A total of 2811 specimens belonging to 14 species from six families were recorded throughout sampling duration. These species were *Spodoptera exigua*, *Spodoptera litura*, *Trichoplusia ni*, *Pseudoplusia includes*, *Helicoverpa zea*, *Helicoverpa armigera*, *Agrotis ipsilon*, *Pieris brassicae*, *Pieris rapae*, *Evergestis rimosalis*, *Manduca sexta*, *Plutella xylostella*, *Lymantria dispar* and *Galleria mellonella*. Spring was rich for lepidopteran populations 64.50 %, followed by winter (23.52), autumn (10.14) and least in summer (1.85). Spring was represented by all the species as mentioned above. *M. sexta* was absent from all the other seasons. *G. mellonella* was not sampled from summer or autumn. The family Noctuidae shared as many as 63.08% in total lepidopteran populations, followed by Pieridae 30.38%, while Pyralidae had the least share 0.68%. Shannon Diversity Index Among Seasons was applied on members of Lepidoptera in different seasons. Significant difference were found between summer and all the other seasons, while non-significant results were found between autumn and winter and also autumn and spring. Canonical Correspondence Analysis (CCA) was applied to check the effect of environmental factors like temperature, humidity,

rainfall and wind speed CCA was performed on lepidopterans collected from four crops. Most of the species showed association with temperature, relative humidity and wind speed.

**COMPARATIVE STUDY ON THE NYMPHAL STAGES OF TWO SPECIES GENUS
SPHINGONOTUS (OEDIPODINAE: ACRIDIDAE: ORTHOPTERA)**

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Immature stages of two species of genus *Sphingonotus* viz. *S. savignyi* and *S. rubescens* has been studied exclusively from Sindh during 2015-2016. Both species pass through six instars and seventh stage become adult stage. Emergence of instars in both species was started from July just after monsoon rainfall, whereas, *S. savignyi* immatures emerge earlier than *S. rubescens* and increases their density rapidly in open ground and rocky area near main field crops. Immature stages were geophiles in nature and usually giving two generations in a year but occasionally third generation of diapaused eggs of *S. savignyi* hatched in winter. A comparative key for immatures of species was also constructed. Instars were occupying almost similar habitat and differentiated through combination of characteristic i.e. coloration, morphometric differences, shape of pronotum and band on femur.

**COMPARATIVE STUDY ON THE IMMATURE STAGES OF *OXYA* (OXYINAE:
ACRIDIDAE: ORTHOPTERA)**

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A rice small grasshopper is considered the major pest in Sindh. It causes million rupees damage annually. Apart from rice it also consumes sugarcane, wheat, herbs, shrubs and grasses in the field. At the present, some observations have been made on the immature stages of three species of genus *Oxya* i.e. *Oxya hyla hyla* (Serville), *Oxya velox* (Fabricius) and *Oxya fuscovittata* (Marschall). During the present investigation it was found that all studied species of *Oxya* comprise on sixth nymphal instars. There are significant differences found among all the early developmental stages of *Oxya* species. In this manuscript all the morphological differences along with illustrations and measurement parameters were highlighted in order to determine the various stages of *Oxya*. Current knowledge on the immatures of these species will be proved very useful for taking control measures at appropriate time.

KEY TO THE ACRIDIDAE ORTHOPTERA FROM PUNJAB PAKISTAN

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Many species of Acrididae is considered most of important pest of many agricultural crops in Pakistan. Their correct identification has been done in this work. At the present an attempt has been made and keys were prepared which based on the easily recognized morphological characteristics for the subfamilies, genera and species of the short horned grasshoppers.

BIODIVERSITY OF SYRPHID FLIES (SYRPHIDAE: DIPTERA) FROM POONCH DISTRICT OF AZAD KASHMIR, PAKISTAN

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Syrphid flies (Syrphidae: Diptera) represent a component of insects that contributes to the plant pollination. This work has studied the abundance, diversity and species richness of syrphid flies at Poonch district of Azad Kashmir, Pakistan. A total of 979 specimens of syrphid flies was collected using Entomological sweep nets and Mailase traps from fruit trees (apples, apricots, peaches, pears and plums) at ten different localities (Alisojal, Banjosa, Datoot, Hajira, Hussainkot, Khaigala, Paniola, Rawalakot, Singola and Topa) of district Poonch. Six species belonging to three subfamilies and five genera were identified. The maximum abundance of *Eristalis tenax* was recorded from Hussainkot and minimum abundance recorded from Rawalkot.

GRASSHOPPER (ACRIDOIDEA) OF GORAKH HILL OF SINDH

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Gorakh Hill is a high altitude (5,688 ft (1,734 meter) plateaus in the province of Sindh facied as a Hill Station. It is located in the Kirthar Mountains 93 km north west of Dadu city or approximately 500 Kilometers from Karachi. Gorakh Hill is spread over 2,500 acres (10 km²) of land. It is very attractive to nature-lovers owing to its temperate weather and beautiful surroundings. Gorakh's elevation gives it a special climate, with sub-zero temperatures during winter and generally below 20 °C in summer, with about 120 mm of average annual rainfall. The Orthopteroid Insects of Gorakh Hill has been studied for the first time. About three hundred specimens were collected in two trips during 2016. The collected material was sorted out into : *Dericorys tibialis* (Pallas, 1773) of family Dericorythidae; *Eremopeza gigaz* (Kirby), of Pamphagidae; *Chrotogonus trachypterus trachypterus* (Blanchard), *Chrotogonus trachypterus robertsi* (Kirby), *Pyrgomorpha bispinosa deserti* (B.Bienko), *Atractomorpha acutipennis blanchardi* of Pyrgomorphidae while from Family: Acrididae: Subfamily Oxyinae, *Oxy hyla hyla* Audinet-Serville, 183, Subfamily Spathosterinae : *Spathosternum prasiniferum* (Walker,

1871), Subfamily Cyrtacanthacridinae: *Anacridium rubrispinium* B. Bienko ;Subfamily (Calliptaminae) *Acorypha glaucopsis* (Walker, 1870), *Sphodromerus undulatus undulatus* (Kirby, 1914); Subfamily Eyprepocnemidinae: *Eyprepocnemis alacris impicta* (Uvarov), *Heteracris littoralis* (Rambur, 1838), *H. adpersa* (Redtenbacher, 1889). Subfamily Oedipodinae: *Acrotylus humbertianus* Saussure, 1884 *Acrotylus* sp. *Aiolopus thalassinus thalasinus* (Fabricius, 1781.), *A. thalassinus tamulus* (Fabricius, 1798), *Hilethera aeolopoides* (Uvarov, 1922), *Locusta migratoria* (L. 1758). *Sphingonotus savignyi* Saussure 1884. *S. rubescens rubescens* (Walker, 1870, *Trilophidia annulata* (Thunberg, 1815 *Scintharista notabilis pallipes* Uvarov). Subfamily Acridinae: *Acrida exaltata* (Walker, 1859) *Duroniella laticornis* (Krauss, 1909), *Phlaeoba tenebrosa* (Walker) *Truxalis eximia eximia* (Eichwald, 1830) Subfamily Gomphocerinae: *Ochrilidia geniculata* (I. Bolivar, 1913) *O. gracilis gracilis* (Krauss, 1902). *O. tibialis*; Bolivar, *Stenohippus mundus* (Walker) *Leva* sp. *Crucinotacris decisa*, *Leionotacris bolivari* (Uvarov) *Scintharista notabilis pallipes* Uvarov. Discovery of these species will prove good addition in exiting fauna of Sindh.

**SOUND PRODUCING ORGANS AND MALE SONGS OF ACANTHOGRYLLUS
BRUNNERI (SAUSSURE) (ORTHOPTERA: GRYLLIDAE)**

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In the present work specimens of *Acanthogryllus brunneri* (Saussure) were recorded for the first time from Tandojam, Sindh. The specimens were described on the basis of their sound producing organs, i.e., the files, teeth numbers and structures and the male songs with its sonogram that separate this taxon from other gryllids. The sound frequency, the stridulatory files and the number of teeth appear to provide some of the most important characters for the identification of males of *A. brunneri*. Previously the members of Gryllidae were identified by their external morphological characters including their male and female genital components. Allard (1910) for the first time recognized the geographical variation in the sounds of field crickets. Diversity of stridulatory organs, signals and behavior play a significant role in separating conspecific species that previously considered comprising the similar one. (Gwynne and Morris, 1986). Due to the advancement in recording technologies, acoustic characters are considered as reliable characters for the identification of gryllids. Specimens of *A. brunneri* were collected from different areas of Tando Jam, Sindh, usually during April to September in summer season. Zoom H4 next recorder was used to record the male songs and slices were prepared with the help of Audacity portable Digital Audio Editor Version 1.2.6. These songs were then observed and analyzed by using the software Praat 5400_win 32. After preservation, the specimens were boiled to soften, then the right tegmen was detached, for photograph by using Nikon Cool Pix 5400 digital camera after placing it under Nikon SMZ 800 Binocular. Structure was observed and analyzed by using Scanning Electron Microscope (SEM) JEOL Japan model No. JSM 6380A, photographs were further analyzed by using the software Coral Draw 13, Coral Inc. 2005.

ABUNDANCE AND DISTRIBUTION OF SCARAB BEETLES (COLEOPTERA: SCARABAEIDAE) IN DISTRICT HYDERABAD, SINDH

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Scarab beetles belong to order Coleoptera (largest order of Insecta) and family Scarabaeidae with approximately 30,000 species of beetles throughout the world, scarab beetles are phytophagous and coprophagous. Adults of many scarab beetles are noticeable due to their relatively large size, bright colors, often elaborate ornamentation, and interesting life histories. Weekly observations were made and the specimens were collected by multiple methods (mercury light trap, pitfall trap and hand picking) result showed that the abundance is correlated with lunar cycle, temperature and physical factors.

EFFECTS OF TEMPERATURE CHANGES ON THE LIFE CYCLE OF MOSQUITO SPECIESFARKHANDA MANZOOR*, HAFSA MEMONA,
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Mosquito-borne diseases are an important public health problem, causes morbidity and mortality worldwide. Mosquito is an extremely synanthropic insect having specific reproductive needs. Their biology and disease ecology are strongly related to environmental conditions such as temperature. Warm temperature and more precipitation events favor spread and arousal of mosquito-borne diseases. This study investigated the differential effects of temperature on the duration of each life stage of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Three strains of adult mosquito were obtained from the Lawrence garden and LCWU, Lahore by trapping techniques and reared at a temperature 28 ± 2 C°, relative humidity $70\% \pm 10$, and a photoperiod of 12h light: 12h dark. Male mosquitos were supplied by sugar solution as long as possible the time for eggs production. Toilet papers were placed regularly to allow mosquitoes landing and feeding without drowning. Females were supplied by blood meals for eggs production via anesthetized rats being placed five to ten minutes into the colonies after shaving their abdominal hairs. Filter papers were placed in the cage for egg deposition. Every new larva was transported to new plastic pans with tap water by use of a plastic pipette into 250 ml glass piker. The egg laying and hatching time for *Aedes aegypti* ranged from 180 to 220 hours at room temperature and between 160 and 170 hours inside the incubator at 28°C. However, egg laying and hatching time for *Anopheles stephensi* ranged from 82 to 90 hours at room temperature and from 72 to 75.5 hours at 28°C. Whereas, time between the egg laying and hatching for *Culex quinquefasciatus* was about 80 hours at room temperature and 70 hours in the incubator at 28°C. Temperature variation effect directly on developmental stages and high rate of development was observed at 28°C in incubator as compared to room temperature. Climate change is expected to affect the life cycle, distribution and seasonal dynamics of mosquito populations, with substantial implications for disease seasonality and persistence. Rising temperatures may increase mosquito population size, development rates and per host biting rate will certainly increases incidence of mosquito-borne diseases.

**TEMPERATURE-DEPENDENT DEVELOPMENT OF *HELICOVERPA ARMIGERA*
(HÜBNER) (LEPIDOPTERA: NOCTUIDAE)**

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Helicoverpa armigera (Hübner) is a notorious pest of different crops and vegetables. The development of *H. armigera* was evaluated against a wide range of constant temperatures (10, 15, 17.5, 20, 25, 27.5, 30, 35, 37.5 and 40°C). The developmental periods of immature stages of *H. armigera* decreased with increasing temperature. The survival rate was also low at extreme temperatures but *H. armigera* failed to survive at 10 and 40°C. Based on the Ikemoto and Takai model the developmental threshold (T₀) and degree days (DD) to complete the immature stages of *H. armigera* were calculated as 11.6°C and 513.6 DD, respectively.

**ESTIMATION OF GENETIC DIVERSITY OF GOMPHIDAE DRAGONFLIES
(ODONATA: ANISOPTERA) OF HAZAR REGION PAKISTAN**

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Current study was conducted on molecular characterization of Gomphid dragonflies collected from Hazara region of Pakistan. A total 345 specimens were collected and identified in to 6 genera covering 9 species. Molecular phylogenetic relationships among members of the family Gomphidae were examined using 735 bp of mitochondrial COI gene and 416 bp of 16S ribosomal RNA gene sequences. Phylogenies of the analyzed taxa were elaborated with maximum likelihood, maximum parsimony and Bayesian analysis. The COI gene and 16S rRNA gene separate and combined COI+16S data sets revealed evolutionary relationship within family Gomphidae at the species and genera level. A total of 1543 nucleotide sequences and 46170 genetic characters/position were used for the COI, 16S and combined COI+16S data set. Mean Pairwise Distances (MPD) of each species were ranged from 0.00 to 37.10%. Evolutionary rate differences among two categories Gamma distribution and Invariant (+G+I) were recorded as 0.06 and 1.20 substitutions per site. DNA based identification using COI, 16S and combined COI+16S data set analyses showed genetic similarities having bootstrap values MLB=70-100%, MPB= 52-100% and BPP=0.75-1%. The analysis of the combined COI+16S data set yielded trees with overall stronger bootstrap support than analyses of either gene alone. Likelihood, Parsimony and Bayesian analyses of the combined COI+16S data set produced well-resolved phylogenetic status of recorded species. The phylogenetic significance of these results is discussed and experimental approaches that would advance our understanding of Gomphidae dragonflies diversity based on molecular study.

**BREEDING BIOLOGY OF RED WATTLED LAPWING
(VANELLUS INDICUS) FROM SOUTHERN PUNJAB, PAKISTAN**

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Current study documented some aspect of breeding biology of Red Wattled Lapwing (*vanellus indicus*) such as breeding season, nest structure, clutch size, incubation period, hatching and fledging success in the district Bahawalpur. We selected 6 sites for the observation; barren, open, cultivated, grassy, stoney along road side and constructed areas with the common vegetation of *Cynodon dactylon*, *Ziziphus mauritiana*, *Albizia procera*, *Cincrus ciliarus*, *Cincrus biflorus*, *Arva jawanica*, *Eucalyptus cameldulensis*, *Acacia nilotica*, *Prosopis juliflora* and *Conocarpus spp.* During breeding season; male selects territory. Breeding season starts from April to June. Both male and female participate in nest formation, incubation and other parental duties. Clutch size was mostly 4 and the mean of the clutch size was 4+0.0 (range 4-4). The incubation period of the eggs was 25-28 days and the average of the incubation period was 27.16+0.477 (range 25-28 days). Total eggs were 24 in 6 nests, out of which 22 were hatched. The percentage of hatched eggs was 91% and the mean hatching rate was 91.66+5.270 per clutch. Fledging success was 79.166+7.682 and the percentage of the fledging was 79%. Predation rate in Red Wattled Lapwing was also observed; found very high due to anthropogenic activities. Study shows that Red-wattled Lapwing is adapting to urban settings and choosing a nest location to minimize human and livestock interference. Current study would provide basic data about Red-wattled Lapwing's breeding behavior that would ultimately helps in its conservation.

**MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF TETRAPONERA
SP. ISOLATED FROM DISTRICT BHIMBER AZAD JAMMU & KASHMIR**

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Man depends upon numerous ecological and economical services offered by countless organisms in natural ecosystem. Among these, insects are the major contributors for ecological and economic values to humans. *Tetraponera* is genus of large, bicolored, arboreal ants' with significant ecological and medical importance. Venom contents of these ants have been reported for antimicrobial, anticancerous activities. The current study employs the identification and phylogeny of *Tetraponera* sp. collected from District Bhimber, Azad Kashmir. *Tetraponera* sp. was identified on the basis of morphological and molecular basis. Morphological analysis was performed using Afrotropical *Tetraponera* identification key. Morphological characters of adult worker ant showed they are bicolored slender body, visible eyes, 12-segmented antennae, distinct abdominal segments and well developed post-petiole showing maximum resemblance to *Tetraponera rufonigra*.

Analysis of cytochrome c subunit 1 (COX1) gene sequence was quite variable and showed 82% homology with *Tetraponera rufonigra*. Sequence analysis of SS rDNA showed 100% homology with *Tetraponera rufonigra*. Phylogenetic analysis based on both COX1 gene and SS rDNA using maximum likelihood and neighbor joining method showed that current *Tetraponera* species is closely related to *Tetraponera rufonigra*.

**INCIDENCES AND DISTRIBUTION OF *CONOCEPHALUS THUNBERG, 1815*
(*CONOCEPHALINAE: ORTHOPTERA*) SPECIES IN DISTRICT MANSEHRA**

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An extensive survey was carried out to collect long-horned grasshopper fauna from Mansehra district. A total of 130 specimens were collected and sort out into two species i-e : *Conocephalus (Anisoptera) fuscus* (Fabricius, 1793) and *Conocephalus (Anisoptera) maculatus* (Le Guillou, 1841). *Conocephalus (Anisoptera) fuscus* (Fabricius, 1793) constructed as new record for this region. Moreover, distribution of species at district level is presented.

**FOOD SELECTION IN THE *CHORTHIPPUS FIEBER, 1852* SPECIES
(*ACRIDIDAE: GOMPHOCERINAE*)**

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Study on food selection in *Chorthippus* species i-e: *Chorthippus brunneus* (Thunberg, 1815) and *Chorthippus biguttulus* (Linnaeus, 1758), was carried out during 2016. The field characteristics of the scheme include remarks on the insect's activities and distribution, tempting experiments, and conclusion of various community studies. *Chorthippus* species are found throughout Hazara division, but is most common in the field with scarce vegetation, some bare ground, and foliage litter. Far ahead it becomes more commonly dispersed, but inclines to avoid the same, and only infrequently enters the nearby woody plants. Laboratory studies resulted that the mouthparts of *Chorthippus* species are of graminivorous type, but in the case of newly hatched and molted instars. Many experiments and nutrition, and records of the field of nutrition, investigates the crop showed a preference for herbs. It showed the limits of food that the insect is able to survive for long periods of time on the plants forbs (broad-leaved weeds), although it is doing when insects have access to herbs. From this it results that species of *Chorthippus* are graminivorous in the field, where they eat fresh flowers and stems of plants and leaves.

MORPHOLOGICAL STUDY AND OPTIMIZATION OF DNA ISOLATION PROTOCOL OF DRAGONFLIES (ODONATA: ANISOPTERA) IN DISTRICT SWAT, KP, PAKISTAN

MUHSIN ALI, SARDAR AZHAR MEHMOOD, WAHEED ALI PANHWAR AND WAHEED KHAN

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An extensive field survey was conducted to collect Dragonflies (Anisoptera) fauna of Swat, during April to June 2016, in the different sites of the study area. A total of 300 specimens identified 11 species in 6 genera, belonging to 3 families. Family Libellulidae having 8 species belongs to 4 genera, while Family Aeshnidae having 2 species belongs to 2 genera and family Gomphidae having 1 species belongs to 1 genus. The family Libellulidae includes the major numbers of species as compare to Aeshnidae and Gomphidae family. The species were *Orthetrum sabina*, *Orthetrum pruinosum neglectum*, *Orthetrum cancellatum cancellatum*, *Acisoma panorpoid panorpoid*, *Trithemis festiva*, *Trithemis pallidinervis*, *Crocothemis nigrifrons*, *Crocothemis erythraea*, *Anax imperator*, *Anax parthenope* and *Gomphidia t-nigrum* were identified. In the present study the most common species was *Orthetrum cancellatum cancellatum* from which 43 specimens were collected. Details for the identification i.e. valid names, their synonyms, measurement of body length, wing span, habitat and morphological description were given. A simple and cheap genomic DNA isolation protocol for dragon fly was developed that will help for further molecular studies in future. It is concluded that there is a vast diversity to explain dragonfly fauna of Swat. Further research should be carried out with larger population size for biodiversity.

COLLECTION AND IDENTIFICATION OF GENUS ANAX (ODONATA, AESHNIDAE) FROM DISTRICT SWAT

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An Extensive field survey was carried out to collect dragonflies of genus *Anax* from Swat during the year 2016. At the present a total of 251 specimens were collected and sorted out into family Aeshnidae genus *Anax* and species *Anax Imperator*, Leach, 1815 and *A. parthenope* (Selys, 1839) respectively. Additionally, morphologically characters along with distributional data are provided.

**BUTTERFLY FAUNA OF ABBOTTABAD DISTRICT,
KHYBER PAKHTUNKHWA PAKISTAN**

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SHABIR AHMED

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Butterflies are one of the most fascinating group of order Lepidoptera. The present study was conducted in Abbottabad during 2016. A total of 160 specimens were collected from 15 localities belong to 05 species, 03 genera and 02 families. Family Nymphalidae species are *Junonia orithya* (Linnaeus, 1758), *Junonia almana* (Linnaeus, 1758) and *Danaus chrysippus* (Linnaeus, 1758). Family Pieridae with *Colias croceus* (Geoffroy, 1785) and *Colotis amata* (Fabricius, 1775) species were recorded. From the present study it was concluded that the *Danaus chrysippus* and *Colias croceus* is the most dominant species. The area has rich fauna of butterflies and recommended for further studies.

**ON DAIMONDBACK MOTH (*PLUTELLA XYLOSTELLA*) FROM
DISTRICT MANSEHRA**

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Plutella xylostella (Linnaeus, 1758) belongs to family Plutellidae was collected from district Mansehra and its incidence and distribution has been recorded at district level. Moreover, highest percentage of *Plutella xylostella* was collected from Mansehra Tehsil i-e 53% followed by Balakot Tehsil with 29% while least percentage was collected from Oghi Tehsil with 18% respectively.

**A NEW RECORD OF GENUS *HETEROPTERNIS* STAL 1873
(ORTHOPTERA: ACRIDIDAE: OEDIPODINAE) FROM PAKISTAN**

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Band-winged grasshoppers belonging to genus *Heteropternis* Stal, 1873 were collected from different localities of Hazara Division during the year 2016. *Heteropternis resprodens* (Walker, 1859) was recorded for the first time from Pakistan. Additionally, photograph along with line drawing is provided for the first time. Finding of present study will contribute to the biodiversity of Pakistan.

**STUDY OF MORPHOMETRIC DIVERSITY IN *A. CERANA* POPULATIONS OF
KPK AND AJK, PAKISTAN**

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The selected bee species *Apis cerana* is native honeybee species of Pakistan also known as “Asian bee” and “Dasi bee”. Thirty samples of worker honey bees were collected from bee hives found in different localities of Peshawar, Charsadda, Mansehra, Dadar and Swat from KPK and Muzaffarabad, Bagh, Rawalakot, Palandri and Poonch from Kashmir region. The bees were preserved in 60% alcohols added with few drops of glycerin before analysis. Thirty one morphometric characters were measured. Body weight (90.5mg), body length (1cm), body width (4mm), cubital index (4um), and number of humuli were found (19) in *A.cerana* species from KPK, Pakistan. Body weight (108.82mg), body width (4mm), cubital index (4.33um), and number of humuli (18) were found in *A.cerana* species from AJK, Pakistan. Length of hind leg showed difference of 8mm and 7.52mm in *A.cerana* from KPK and AJK respectively. Number of humuli and cubital index were appeared most important characteristics in discrimination of worker bees from ecologically different niches. Mean width of basitarsus nearly same (0.59, 0.58) in *A.cerana* of KPK and AJK, Pakistan. Mean width of 2nd tergite (2,2), 3rd tergite (2,2.17) and 4th tergite (2,2.01) found in *A.cerana* of KPK and AJK, Pakistan respectively. The length of foreleg (6,6.4mm), middle leg (7,7.4mm), length of 2nd, 3rd, 4th, tergites (7mm,8mm) in the *A.cerana* were approximately same in all worker bees collected from two regions KPK and Kashmir region of Pakistan.

**TAXONOMIC STUDY OF GENUS *JUNONIA* (HUBNER, 1819)
(NYMPHALIDAE: LEPIDOPTERA) FROM SINDH**

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The genus *Junonia* Hubner belongs to the family Nymphalidae and order Lepidoptera. They are medium sized and having reduced fore legs and covered with hair, ocelli of different colors. The genus *Junonia* Hubner are worldwide distributed and fly for a long distance and carry pollen grains along with their legs from flower to flower which help in the cross pollination. The present study was carried out from Sindh during January 2015 to December 2015. A total 123 specimens were collected from different localities of Sindh. The identification was done through the literature. Four species of genus *Junonia* Hubner namely *Junonia almana* L., *Junonia hierta* F., *Junonia lemonias* L. and *Junonia orithya* L., belonging to family Nymphalidae were identified. The species are described on the basis of morphological characters (coloration of wings, structure of mouthparts, maxillary palpi) and internal male and female genitalia. For examination of the internal male and female genitalia, the abdomen of the specimens was removed from the body and boiled in 10% potassium hydroxide (KOH), then dissected from the lateral side with the help of the forceps. Genitalia was separated from abdomen and washed in tap water then examined under the

dissecting microscope. These species of genus *Junonia* Hubner are first time recorded from Sindh, Pakistan.

**FAUNISTIC STUDIES OF GRASSHOPPER OF DISTRICT SWABI,
KHYBER PAKHTUNKHWA, PAKISTAN**

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This study was conducted to know about the grasshopper fauna of district Swabi. Sampling was done from four tehsils of district Swabi. i.e. Swabi, Topi, Lahor and Razar. Each tehsil had three collection points. From tehsil Swabi collection was done from Shamansor, Anbarand Kunda and from the famous graveyard of Swabi. From Topi: Jadoon, Bamkhel and Ghazi were the collection site and from lahor specimens were collected from Manki, Hand and Haryan. From Razar the collection site was Dagi, Yarhussainand Ghohatee. The sampling was done regularly two to three times in a month. The sampling timing was as at morning and sampling was done on first and fifteen date of each month. I collected 334 specimens from the district Swabi from their four main tehsils. Specimens were identified by using key described in Shahid (1964) and we found 28 species, 18 genera, 10 subfamilies and 3 families namely as acrididae, pygomorphidae and tetrigidiae.

**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, structure of eggs and egg pods, and effect of temperature on the their development of different instars were studied. A total 820 specimens including various nymphal instars and adults (MALES AND FEMALES)were collected from different localities of district Dadu such as Dadu city, Phulji, village Hafiz Meer Mohd Kalhoro, village Bhawalpur, Village Bachal Bouk, village Syed Naban Shah and Village Loung Khan Lund during the year 2015-2016.

**BIO-DIVERSITY OF SHORT HORNED GRASS HOPPERS
(ACRIDITAE) OF MIDDLE SINDH**

ABDUL SATTAR KALHORO AND PROF: NAHEED BALOCH
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Short horned grass hopper of middle Sindh area major pest of crops, cotton, wheat, sugarcane, grasses, rice etc. The short horned grass hoppers are very important for eco system, food chain and pyramids, There are four zones of middle Sindh mountain zone, desert zone, reverie belt and agriculture crops. It is very important to study about its pest insect and its bio diversity which will help in various fields of biological sciences.

**PRESENCE OF *ARGIOPE LOBATA* PALLAS, 1772 IN THE CHABBAR SYEDAN,
DISTRICT JHELUM, PUNJAB, PAKISTAN**

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Spiders are integral part of the ecosystem, playing the role of bio-pesticide chiefly and has adopted to almost all kinds of ecosystems. These constitute a diverse group and variety is enormous. Genus *Argiope* fall in the large sized spiders which have diverse distribution even reached the Pakistan and India (Indian subcontinent), represented by *Argiope aemula*, *Argiope catenulate*, *Argiope pulchella*, *Argiope anasuja* and *Argiope lobata*. In Pakistan, their distribution has not been fully recorded and many remote areas are unknown to naturalists/entomologists/arachnologists. One of these areas is Chabbar Syedan, present at the base of Bakrara ridge. In this article eight specimens of *Argiope lobata* have described and reported photographically in their natural habitat and environment for the first time from this area to extend the knowledge of its distribution in Pakistan.

**PREVALENCE OF SCABIES IN NEW CENTRAL JAIL AND IN A
RESIDENTIAL AREA OF MULTAN**

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A prospective study was carried out both at central jail and a residential area of Multan in April 2008 to estimate the prevalence of *Sarcoptes scabiei* infestations with a focus on sex, age, caste and socio-economic factors. Out of 1146 population 130 (11.34%) had scabies. The age of the hosts with scabies was ranging from 1 to 100 years. The percentage of this infection among males was 11.51% (124positive/1077 cases), and among females was 8.6% (6/69). Prevalence was higher for males than females. Prevalence of scabies was found to be highly associated with age. The highest prevalence 22.6% of parasite was recorded in age group > 20 years. The prevalence was highest in those aged up to 20 years. While the lowest prevalence 9% was recorded in age group 21-30 years. Caste wise prevalence was also calculated, highest prevalence was found in Jutts (43.1%) while lowest in Maliks (14.2%). In addition scabies infestations were more prevalent in lower class with low income (16.25%) and less prevalent in upper class (5.88%).

**DISTRIBUTION OF BUTTERFLIES (LEPIDOPTERA: RHOPALOCERA)
IN DISTRICT SWABI, KHYBER PAKHTUNKHWA**

MUHAMMAD BASIT ALI AND MUNAWWAR SALEEM AHMAD

This study deals with the Butterfly (Lepidoptera; Rhopalocera) fauna of district Swabi Khyber Pakhtunkhwa. Collection of butterflies was done in 12 localities of district Swabi from April to July 2016. These localities are: Ismaila, Adina, YarHussain, Punjpir, Anbar, Dagai, Maneri, Shewa, Shah Mansoor, Utlā, Beer Galai, Malak Abad(Gadoon),. Total 374 specimens were collected from different crops, grasses and general vegetation. The identification revealed that there are thirty- one (31) species of butterflies under twenty- four (24) genera and five (5) families in Swabi. The families include Pieridae, Papilionidae, Nymphalidae, Hesperidae and Lycaenidae. In Pieridae seven (7) genera having eight (8) species was found. In Papilionidae two (2) genera having six (6) species. In Nymphalidae twelve (12) genera having fifteen (15) species. While in Lycaenidae two (2) genera having two (2) species and in family Hesperidae one (1) genera having one (1) species.

**QUALITATIVE ANALYSIS OF SECRETIONS OF
TETRAPONERA SP. BY GC-MS**

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Ants of genus *Tetraponera* are known to form mutualistic relationship with their host tree. They get shelter in the trunk of host tree and in return protect it from pests and other mammals with very painful sting. This makes them a viable candidate for biological control and integrated pest management (IPM). In current study *Tetraponera* were collected from Barnala, Azad Kashmir. Their body secretions were extracted by using hexane as a solvent. Sample was purified by Flash Chromatography using acetone as eluent and fractions were subjected to GC-MS. Compounds resembling with 3-undecanone and 3-octanone were found in fraction 1 (40% acetone) which are reported to induce biting behavior and act as attractant in *Crematogaster* sp. and *Myrmica* sp. respectively. Octyl hexanoate and octadecyl butyrate resembling components were detected in fraction 2 (60% acetone). These are known to work as repellent in *Formica cunicularia* and as slave maker in queens of *Polyergus rufescens*. Compounds showing similarity with 4-methyl-3-heptanyl oleate, isogeraniol and 9, 10-epoxynonadecane were found in fraction 3 and 4 (80% and 100% acetone) which are known to protect volatile pheromones and compounds and cause them to last longer. Fraction 4 also contained organic acids; heptadecanoic acid, tetradecanoic acid, octadecanoic acid, hexadecanoic acid and oleic acid. Compound similar to 2-methyl-1-hexanol was also found which is reported to have anti-microbial activities in *Cataglyphis* sp. This fraction also contained component resembling with dihydroactinodiolide was also detected which is known to act as marker in *Solenopsis invicta* (Fire ants).

**EFFECT OF DIFFERENT LARVAL DIETS AT VARIOUS WATER DEPTHS
FOR THE SEXUAL DIMORPHISM OF *Aedes aegypti* UNDER
LABORATORY CONDITIONS**

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Dengue is a reportable disease in Pakistan with alarming spreading potential from central parts of the country towards Northern areas (Malakand Division) and therefore, need proper attention. The use of insecticides for vector control is the only option presently in place. But due to environmental constraints, health hazards and resistance development in mosquitoes, other environment friendly vector control strategies with main emphasis on Sterile Insect Technique (SIT) are needed. Sex separation of *Aedes* mosquitoes by inducing/enhancing dimorphism at pupal stage through nutritional means may provide opportunities for future launching of SIT programs in KPK and finally in Pakistan. The effect of different larval diets; IAEA, NIFA and Stevia at different water depth were tested for the sexual dimorphism of *Aedes aegypti* at the pupal stage as a pre-requisite of SIT program of the dengue vectors. The experiments were conducted at Nuclear Institute for Food and Agriculture (NIFA), Peshawar during November, 2016. The diets were blended and strained through 50 and 75 mesh size strainer for the subsequent formulations. Each treatment was replicated three times. Larval density (30 larvae) were tested at six different water depth in plastic cups as 0.5, 1, 2, 3, 4 and 5 cm and 3% concentration of each diet. The results showed the maximum size of female as 12.73 mm in stevia treatment followed by IAEA and NIFA as 7.99 and 7.93 mm, respectively. Non significant results were observed for each water depth on the size of male mosquitoes pupae. It was concluded that the stevia diet showed the significant effect on sexual dimorphism and can be exploited for the production of distinct sexual dimorphism in male/female pupae of *Aedes aegypti*.

**ANT DIVERSITY (FAMILY FORMICIDAE) IN DISTRICT CHARSADDA,
KHYBER PAKHTUNKHWA, PAKISTAN.**

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The present study was conducted to investigate the diversity of ants at district Charsadda, Khyber Pakhtunkhwa, Pakistan, which was the first study in District Charsadda and second one in Pakistan since 1947. For this purpose, ants were collected from Indoors (Rooms, kitchens, washrooms) outdoor (streets, crop fields, barren grounds, graveyards) sources. Random sampling was done using buccal aspirator, pitfall traps and hand picks methods for the collection of ants from the three tehsils (Charsadda, Tangi, Shabqadar) of district Charsadda. All samples were examined for the study of ants diversity. The identification of ants species was undertaken at, Entomology laboratory, Department of Zoology University of Peshawar. A total of 19 species representing 11 genera and 3 subfamilies namely Formicinae, Myrmicinae and Dolichoderinae, were identified during the study. Myrmicinae had the most dominant subfamily with the presence of 11 species (*Crematogaster subnuda*, *Meranoplus bicolor*, *Messor instabilis*, *Monomorium aberrans*, *Monomorium dichroum*, *Monomorium indicum*, *Pheidole binghami*, *Pheidole fergusonii*, *Pheidole indica*, *Pheidole latinoda*, *Pheidole sulcaticeps*). Genus *Pheidole* has maximum number of species

(five) followed by *Camponotus* and *Monomorium* with three species each. All the rest of the genera were represented by one species each. All of the recognized species (19) had reported for the first time from the district Charsadda. Among the 19 recognized species, 13 species are first time reported from Pakistan.

SECTION – IV**P A R A S I T O L O G Y****ANTILEISHMANIAL ACTIVITY OF ALKALOIDS ISOLATED FROM
BERBERIS GLAUCOCARPA AGAINST PROMASTIGOTES OF LEISHMANIA
TROPICA CLINICAL FIELD ISOLATE KWH₂₃**

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In the present study, 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 KWH₂₃. Four different concentrations of each compound and one negative control were prepared and were placed in two 96-well microtitre plates containing 1×10^5 *Leishmania tropica* Promastigotes/well. The stock compounds concentration was 400µg/mL of Dimethylsulfoxide (DMSO) and was used in 100µM, 75µM, 50µM, and 25µM 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 (IC₅₀) as evaluated in GraphPad Prism 5 Software was 0.7547µM, 0.7563µM, 0.7544µM, 0.7319µM, 0.7520µM, 0.7563µM, and 0.7563µM 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.

**IDENTIFICATION AND INFESTATION OF ECTOPARASITES TICKS, MITES AND
LICES ON CHICKEN (*GALLUS DOMESTICUS*) IN DISTRICT KHAIRPUR,
SINDH PAKISTAN**

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The poultry industry occupies an important position in provision of animal protein such as myoglobin and egg albumin and generally plays a vital role in national economy as a revenue provider. Ectoparasitism is an important factor associated with poor production of village

indigenous chickens and negatively affects the productivity potential of the local free-range chickens since they either compete for feed or cause distress to the birds. Biting lice or Chewing lice (*Phthiraptera: Amblycera, Ischnocera*) are important poultry ectoparasites living mainly on the skin of chickens, may cause irritation of the skin, restlessness, overall weakening, cessation of feeding, loss of weight, inferior egg laying capacity, and skin lesions that may become sites of secondary infection are most pathogenic causing anaemia and heavy multi-focal skin lesions or even death of infested birds. Therefore, the proposed study will help ful to provide data regarding percentage prevalence, and Identification and Infestation of Ectoparasites Ticks, Mites and lice on Chicken (*Gallus Domesticus*) in District Khairpur, Sindh Pakistan, which will ultimately be utilized by the veterinarians and other researchers in the study of the these ectoparasites insects infesting Pakistani poultry.

NEMATODE PARASITES RECOVERED FROM HOUSE MOUSE, *MUS MUSCULUS* (RODENTIA: MURIDAE) OF DISTRICT NOSHEHRO FEROZ, SINDH, PAKISTAN

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During current study on the nematological studies of House mouse, *Mus musculus* (Rodentia: Muridae), a total of 32 hosts were collected from residential areas of district Noshehro Feroz and dissected for the presence of helminthic infection. Results revealed 72% infection of nematodes. Amongst these, only two species of nematodes including *Syphacia muris* Yamaguti, 1935 and *Aspicularis tetraptera* Schulz, 1924 were identified. Both species of these nematodes are previously reported worldwide in rats and are being reported second time from Pakistan.

HISTOPATHOLOGICAL CHANGES IN THE INTESTINE OF DUCK (*ANAS PLATYRHYNCHOS* LINNAEUS, 1758) DUE TO CESTODE INFECTION

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Histopathological changes caused by cestode in the intestine of duck (*Anas platyrhynchos*). During the present study the intestine of *Anas platyrhynchos* was found to be infected with cestode *Dicranotaenia coronula*. Histopathological changes revealed inflammation, fibrosis, hypertrophy, hyperplasia and degeneration especially in the areas where parasite physically present. The technique of Microtomy was utilized to study this changes produced by cestodes (*Dicranotaenia coronula*) in the intestine.

**NEW HOST RECORD OF CESTODES FROM DUCK
(ANAS PLATYRHYNCHOS) FROM PAKISTAN**

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Three species of cestodes are recovered from the intestine of mallard (*Anas platyrhynchos*), which were identified as *Schistocephalus solidus*, *Dicranotaenia coronula* and *Haploparaxis endacantha*. These cestodes demonstrated for the first time and it marks the first record of duck helminths in Karachi, Pakistan. Seven specimens of *S. solidus*, five specimens of *D. coronula* and six specimens of *H. endacantha* were recovered from the intestine of mallard. *S. solidus* basically found in fish gut but also invade the intestine of fish-eating birds or aquatic birds. Majority of the ducks which were examined carried a single worm species but mix infections were also recorded.

**TWO NEW SPECIES OF GENUS GANEO (DIGENEA: TREMATODA:
LECITHODENDRIIDAE) FROM FROG RANA TIGRINA (DAUDIN, 1803)
IN KARACHI, PAKISTAN**

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Frog population has declined worldwide, although they play an important role in our ecosystem. In the present study two new species of genus *Ganeo* Klein, 1905 are below described from the intestine of the frog *Rana tigrina* (Daudin, 1803) from Karachi, Pakistan. *G. jonesae* new species is characterized by having elongated, aspinose body, oral sucker small; pharynx well developed, esophagus moderate size, caecal bifurcate at middle of cirrus pouch and reaching to posterior third of body region. Ventral sucker, small, median, post bifurcate; testes diagonal; cirrus pouch simple containing internal seminal vesicle; ovary small, post-testicular; region of cirrus pouch to the hind body. Eggs small, oval and numerous. *G. elongatum* new species is characterized by having elongate body, oral sucker, small, terminal; esophagus long; caeca of moderate length reaching to posterior third of the body; testes two oval, diagonal, pre-ovarian, intercaecal, lateral to cirrus pouch; ovary is post-testicular, almost equal in size to testes; ventral sucker small, intercaecal, pre-ovarian; cirrus pouch clavi form with well developed external seminal vesicle; vitellaria as compact mass in mid lateral region reaching to intercaecal region; Eggs small, oval and numerous.

**PREVALENCE OF FASCIOLA INFECTION IN SHEEP IN AND AROUND
RAWALAKOT-AZAD JAMMU & KASHMIR**

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Fasciolosis is wide spread parasitic disease of herbivores results in economic losses in livestock appearing in the form of mortalities, infected livers, slow growth and reduction of milk, meat and wool production. This study was designed to find out the prevalence of ovine Fasciolosis in and around Rawalakot A.J.K. A total of 300 faecal samples were randomly collected from sheep of different age groups and of both sexes directly from rectum in and around Rawalakot. Faecal samples were processed for qualitative examination. The qualitative examination of faecal samples was done by direct microscopic examination, sedimentation and centrifugal floatation methods. The data collected was analyzed by applying Chi-square test to check the prevalence of ovine Fasciolosis. Out of these, 101(33.6%) animals were positive for Fasciolosis through coprological examination. The results demonstrated a 34.6% prevalence of Fasciolosis in female sheep and a 30.6% in male sheep. There was no significant difference in prevalence of Fasciolosis based on sex of animal. Significant difference of prevalence ($P < 0.05$) was found amongst sheep of different age groups. It was concluded that Fasciolosis is prevalent in sheep in and around Rawalakot Azad Jammu & Kashmir and is continuous threat to the health and production of animals.

**DESCRIPTION OF NEW SPECIES *CONSPICUUM QURATULAINI* N.SP. (TREMATODE:
DICROCOELLIDAE) FROM COMMON MYNA *ACRIDOTHERES TRISTIS*
(PASSERIFORMES: STURNIDAE) IN DISTRICT LARKANA,
SINDH, PAKISTAN.**

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A new trematode *Conspicuum quratulaini* n.sp. is recorded in the Gall bladder of Common myna *Acridotheres tristis* of District Larkana, Sindh, Pakistan. In all, 04 trematodes were recorded. Standardized methodology was exploited to mount the worm permanently. The new species is characterized by having elongated, thick and highly muscular body; anteriorly rounded; posteriorly also rounded but reflects wrinkle type appearance; oral sucker bean shaped and sub-terminal; pharynx long slightly bean shaped but completely separated from the oral sucker; esophagus not visible; ventral sucker is smaller than that of oral sucker but globular in shaped; both testes are asymmetrical overlapped with uterus, left testis smaller than right testis and oval in shape; the worm contains dimple at the lateral side; ovary oval in shape and located behind the left testis;

vitelline follicles commencing at the level of testicular zone; ceaca not observed due to jumbling of uterus; small spaces manifesting empty area, whereas rest of the body is filled with uterus; eggs are operculated and brownish in colors measuring correspondingly. Present recorded trematodes reflect diversification from their congeners in body shape; size; presence of shoulder type outgrowths; morphologically wrinkle appearance; distribution of uterus, post-testicular space and shape of testes. On the basis of such morpho-metrical changes this species refers to be new addition to the taxonomical domain hence it is treated as a new species. This new species is dedicated in the honor and name of (Dr. Quratulain Soomro Medical officer).

STUDY OF ROUND WORM (*ASCARIS LUMBRICOIDES*) OF BHAN SAYEEDABAD AND ADJOINING AREAS IN SCHOOL CHILDREN (FEMALES)

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The parasite which is common in area Bhan Sayedabad and its near adjoining area among school children (Females) which cause infection and other problems of health. It is necessary to study and diagnose its impact on school children (Females) under teenage.

***STEPHANOPRORA ALYKHANI* SP.N. (TREMATODA: ECHINOSTOMATIDAE) FROM THE INTESTINE OF *VANELLUS INDICUS* (REDWATTLED LAPWING) IN LARKANA, SINDH, PAKISTAN**

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During current studies on the helminth parasites of *Vanellus indicus* (Redwattled lapwing) were collected from District Larkana, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. Out of three, two were infected with three specimens. The worms were mounted permanently according to standard procedure for further detail study and identified as belonging to genus *Stephanoprora* Odhner, 1902 and proposed as *Stephanoprora alykhani* sp.n. The new species is characterized by having: body elongate, head collar small with 26 spines, oral sucker terminal, esophagus moderately long, acetabulum larger than oral sucker, rounded and highly muscular, ovary median, pretesticular oval to rounded, testes median, tandem situated in the middle of the body, cirrus pouch plump shaped, vitellaria shorts, extending with loops between the ovary and acetabulum. The species name is in honor of Dr. Aly Khan, Principal Scientific Officer and Director, Crop Diseases Research Institute, Southern Zone Agricultural Research Centre, (Pakistan Agricultural Research Council) Karachi University Campus, Karachi, 75270 who has published several research papers dealing with Plant and Animal Parasites and has prepared several 'Patents' for control of Plant parasitic nematodes in Pakistan.

**ECTO-PARASITES INVESTIGATION OF ROSY STARLING IN
DISTRICT MUZAFFARGARH**

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The study was conducted on ecto-parasites of migratory rosy starling in some selected crops of district Muzaffargarh. A total of 120 samples were investigated within five months. Birds were captured by walk in Trap and Mist Net methods. Ecto-parasites were collected and transferred to 10% formalin solution. The identification and analysis of ecto-parasites were conducted in parasitological laboratory of University of Agriculture Faisalabad. A total of 96 ecto-parasites specimens were collected from birds and identified on morphological bases. About fifty two percent rosy starlings were found infested with at least one ecto-parasite. A total of seven lice, one mite, one bed bug and one fire ant species of ecto-parasites were identified namely: *Menopon gallinae*, *Lipeurus caponis*, *Chelopistes meleagridis*, *Felicola subrostratus*, *Goniocotes gallinae*, *Sturnidoecus affinis*, *Pseudolychia canariensis*, *Cimex lectularius*, *Solenopsis invicta* and *Ornithonyssus bursa* respectively. The Shannon diversity index (H') for ecto-parasites was 2.1770 evenness was 0.9455 and richness was 10.000. The diversity index (H') was higher 1.969 for birds captured from Jatoi, whereas least H' (1.525) for birds of Ali Pur. Relative abundance of *Solenopsis invicta* (17) was highest while for **Ornithonyssus bursa** (3) was least. The prevalence of ecto-parasites was high (40.625%) in the month of July while it was least (9.375%) in August which indicates that they brought least infestation from the regions they arrived but got infected from local sources.

**SERO-PREVALENCE OF ECHINOCOCCOSIS (*ECHINOCOCCUS GRANULOSUS*) IN
CAMEL POPULATION OF SELECTED ECOLOGICAL ZONE OF
BALUCHISTAN, PAKISTAN**

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Among parasitic problems of *camelina*, Echinococcosis (*E. granulosus*) is a worldwide zoonosis and one of the leading causes of hydatid cyst. The hydatid cyst is an important zoonotic disease leading to economic losses in terms of mostly cysts formation on internal organs liver and lungs. It is an endemic worldwide which affects a large no of human and animal population, mostly in under developing countries. *E. granulosus* 8 strains have been discovered till now G1, G2, G3, G6, G7, G8, G9 and G10 on DNA based characterization and morphology. *E. granulosus* strain G6 is specific to camel. It requires two hosts the final and an intermediate host for completion of its lifecycle. Dog is a definitive host for this parasite and sheep, goat, cattle and camels are its intermediate hosts. Prevalence estimates of Echinococcosis are recorded in sheep, goat and cattle however no data are available on the magnitude of the disease in camel population of Pakistan. Therefore, the proposed study has been planned to investigate the sero-prevalence on the basis of,

age, sex and risk factors of Echinococcosis in selected camel population area of Baluchistan. For this Purpose 350 blood samples has been collected in gel activator and EDTA tubes from different ecological zones of Baluchistan and serum was separated on centrifugation (5000rpm for 10 minutes). All samples will be saved in cryovials at -20 °C in the Department of Parasitology, University of Agriculture Faisalabad for serologic analysis. The animals and environment data was also registered on a questionnaire. The samples will be tested using ELISA test for *E. granulosus*.

STUDIES ON TREMATODES OF SOME BIRDS OF DISTRICT MANSEHRA, KHYBER PAKHTUNKHWA, PAKISTAN

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During the current study, a total of 60 birds (crow, myna and magpie) were collected randomly from different Tehsils of district Mansehra, KP, Pakistan, and brought to the parasitology laboratory, Hazara University, Mansehra. The visceral organs like stomach, small intestine, large intestine, liver and lungs of the birds were removed and examine for the presence of trematode parasites. Two different Dicrocoeliine trematodes were recovered from two different hosts. Five specimens of *Lyperosomum longicauda* (Rudolphi, 1809) Looss, 1899 were recovered from the liver of house crow, *Corvus splendens*. Two specimens of *Zoonorchis jonesae* sp. n were collected from the bile ducts of Rufous Treepie, *Dendrocitta vagabunda*, Latham, 1790. The new species is characterized by elongated body with maximum width at the region of acetabulum; oral sucker much smaller than acetabulum; genital pore immediately behind caecal bifurcation; testis symmetrical in position, round to slightly oval, posterolateral to acetabulum; ovary round sub median, post-testicular; Vitellaria commencing from the level of the middle of the ovary and uterine coils mostly present in post testicular region occupying the whole body. The genus *Zoonorchis* Travassos 1916 is being reported for the first time in Pakistan. The species appear different from all the previously reported species of the genus *Zoonorchis* Travassos 1944. The new specie is named in the honor of Dr. Arlene Jones, United Kingdom.

REPRODUCTION AND PATHOGENICITY OF *MELOIDOGYNE INCOGNITA* ON FIVE CHILI CULTIVARS

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Root-knot nematodes are the most widely distributed, destructive plant pathogens and cause colossal yield loss. The losses are considered to be influenced by inoculum levels and type of cultivar. In the present studies effect of different inoculum levels (0, 500, 1000, 2000, 5000) of *Meloidogyne incognita* was investigated on five chili cultivars (High Fly, PV-VI, Revival, Kot Sultan, Skyline II). All the inoculums levels varied significantly in causing reductions in growth

parameters. The reduction in growth parameters increased with an increase in inoculum levels showing a direct relationship between inoculum levels and growth parameters. The inoculum levels also affected nematodes infestations. Number of galls and egg masses increased with an increase in inoculum levels; being minimum at lowest level and maximum at highest density. On the other hand, fecundity and rate of nematode build up was found to be the maximum at the lowest level. As the level increased significant increase in these parameters were observed. Inoculum levels behaved differently on different cultivars. Maximum reductions in growth parameters, nematode infestation and reproduction of *M. incognita* were observed in case of Highfly. The cultivar Skyline-II was the least affected. None of the cultivars was found to be immune or resistant against *M. incognita*. The cultivar Skyline-II was found to be susceptible and the remaining cultivars were highly susceptible.

**FREQUENCY DISTRIBUTION OF GASTROINTESTINAL
HELMINTHS AMONG DIFFERENT DOMESTIC BREEDS OF GOATS
IN FAISALABAD DISTRICT**

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Domestic goat (*Capra hircus*) is a multiuse animal which plays useful role in the livelihood of farmers. Gastrointestinal (GI) helminths pose serious threats to goat population as they cause production losses in terms of high morbidity, mortality, cost of control and treatment. Epidemiological studies and breed resistance to GI helminths is an important tool to minimize the economic losses due to GI helminths in small ruminants. In the present study, 3096 faecal samples from different goat breeds were screened through McMaster egg counting technique for determining the prevalence of GI helminths in Faisalabad district for a calendar year. With an overall 65.60 % prevalence of GI helminths in goat population, the distribution of nematodes, trematodes and cestodes were 55.65%, 6.23% and 3.71%, respectively. Recorded species of helminths were: *Haemonchus contortus*, *Trichostrongylus* spp, *Ostertagia circumcincta*, *Chabertia ovina*, *Oesphagostomum* (*O.*) *columbianum*, *Fasciola hepatica*, *Marshallagia marshalli*, *Bunostomum trigonocephalum*, *O. radiatum*, *Moneizia expansa*, *Trichuris ovis* and *Strongyloides papillosus* in descending order of abundance. Breed, age, floor pattern, animal keeping and feeding system were found statistically associated ($P < 0.05$) with the prevalence of GI helminths in goats. Gender and housing system were not found associated ($P > 0.05$) with the prevalence of GI helminths. The present study provided the prevalence of GI helminths along with associated risk factors which will help in selective breeding of the resistant goats as an indirect way to reduce the abundance of GI helminth population in goats.

FREQUENCY DISTRIBUTION OF ECTOPARASITIC FAUNA INFESTING THE DOMESTIC DOG POPULATION OF DISTRICT RAWALPINDI, PUNJAB, PAKISTAN

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The current study was conducted to determine the frequency distribution of ectoparasitic fauna infesting the domestic dog population of district Rawalpindi, Punjab, Pakistan. In this regard, a passive surveillance was conducted for a period of nine months from October, 2015 to June, 2016 by using sentinel units like farms, veterinary hospitals and pet clinics to identify the infested dogs through clinical examination. The data was recorded on a pre-designed questionnaire. All the ectoparasitic specimens were collected from animals with help of forceps and transferred to McCartney sample collection bottles containing 70 % ethanol as a preservative. Morphological characterization of ectoparasites was carried out under stereoscopic microscope using standard keys. Overall prevalence was found 14.59% (448/3070). Among various ectoparasites, ticks (33.58%) were found predominant followed by lice (9.58%), fleas (6.84%) and mites (3.23%). *Hyalomma anatolicum* (20.39%), *Rhipicephalus microplus* (13.18%), *Ctenocephalides felis* (4.35%), *Ctenocephalides canis* (2.48%), *Haematopinus* spp. (3.48%), *Damalinia* spp. (3.23%), *Linognathus* spp. (2.73%), *Psoroptes ovis* (2.23%) and *Sarcoptes scabiei* (0.99%) were identified species of ectoparasites. Age, sex and breed of host showed no statistical association ($P > 0.05$). Among various husbandry practices, floor pattern was significant ($P < 0.05$), while feeding system, housing system and animal keeping were not found significantly ($P > 0.05$) associated with the prevalence of ectoparasites. Seasonal trend showed highest prevalence of lice and mites during the months of winter while peak frequency distribution of ticks and fleas was recorded during summer and spring months respectively. The fallout of the present study may play significant role for planning an effective control of ectoparasites in the study area.

PREVALENCE OF INTESTINAL NEMATODES AND HAEMOPARASITES IN DOMESTIC AND STRAY DOGS

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Gastrointestinal helminthes and haemoparasites pose a serious threat to the morbidity and mortality of pet and stray dogs. The present study was aimed to assess these two major parasite groups in pet and stray dog population of Faisalabad city. For this purpose, blood and fecal samples were collected from the pet and stray dog population of Faisalabad. Among selected dogs, Bull dogs were in greater number then in descending order were German shepherd and Labrador. Based on different hematological techniques, about 18% of examined dogs were found positive for

the presence of only one protozoon species babesia and not a single dog was found positive for other haemoparasitic diseases (Dirofilariasis, Ehrlichiosis and Trypanosomiasis). Regarding prevalence of helminthes, about 77% dogs were found positive for the presence of helminth eggs ranging from mean eggs per gram of feces 200 to 1450. Prevalence of nematode species was significantly high than other helminth's groups cestodes and trematodes. The common reported species of helminthes were *Ancylostoma braziliense*, *Necator americanus*, *Toxocara leonina*, *Dipylidium caninum*, *Linguatula serrata* and *Oncicola canis*. Influence of age was observed on the infection level and it decreased with the increase in age of pet dog. Various breeds of dogs showed different infection level. Regarding infection level, Pointer dogs were found highly resistant to nematodes and Russian were highly susceptible. Infection level of bull dog, labrador and Doberman was almost at the same level ($P > 0.05$) but overall difference among breeds regarding proportion of helminth infection was significant ($P < 0.05$).

COMPARITIVE ENDOPARASITIC ANALYSIS AT SELECTED CAPTIVE LOCALITIES OF ANTILOPE CERVICAPRA 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 deworming but we were not sure that each individual in the herd was taking proper dose of dewormer. There is need to revise the de-worming protocol otherwise already threatened animal would face more troubles and to spend healthy life. It is recommended antelopes kept at Lal Sohanra National Park should also be given proper dewormer along with natural wormicides

**DAMAGE FUNCTIONS OF SOUTHERN ROOT-KNOT NEMATODE
(*MELOIDOGYNE INCOGNITA*) ON CUCUMBER**TARIQ MUKHTAR,^{1,*} MUHAMMAD ZAMEER KAYANI² AND
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Southern root-knot nematode (*Meloidogyne incognita*) is one of the major constraints to cucumber production throughout the world. The damage functions of *M. incognita* on cucumber have not been studied; ergo, in the present studies the effects of a geometric series of five initial population densities of *M. incognita* were investigated on growth and yield parameters of cucumber. The relationships among plant ages at inoculation, growth and yield parameters, and initial nematode density were also determined. Cucumber plants of 2-, 3- and 4-week ages were inoculated with different nematode densities and observations were recorded 9 weeks after inoculation. Reductions in growth and yield parameters by nematode densities were calculated over control. All inoculum densities and ages of plants at the time of inoculation influenced growth and yield of cucumber. It was observed that all inoculum levels caused significant reductions in these parameters and were found to be negatively correlated with the latter. On the other hand, ages of plants at inoculation had positive correlations with these parameters at each inoculum level. The results demonstrated that *M. incognita* has the potential to severely impair the growth of cucumber and by delaying early exposure of the latter to nematodes can significantly abate yield losses.

**NEMATODES INFECTING APPLE AND THEIR POPULATION DYNAMICS IN THE
POTHOWAR REGION OF PAKISTAN**

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Apple, an important tropical fruit, is being grown in the Pothowar region of Pakistan. In the present studies infestation of plant parasitic nematodes were seen on this fruit and their population dynamics was studied. A total of six nematodes were recorded with varying populations. The overall prevalence of *Pratylenchus* spp. and *Tylenchorhynchus* spp. was found to be 69% followed by *Meloidogyne* spp. The prevalence and populations of other nematodes were low. In case of *Helicotylenchus* spp. prevalence of 29% was observed for Golden while it was 20% in King Amri. As regards *Hoplolaimus* spp. maximum prevalence of 60% was recorded for King Amri while it was only 14% for Golden. Similarly, the prevalence of *Xiphinema* spp. was 29 and 20% for Golden and King Amri respectively. The prevalence of these three nematodes on Star King was not observed. High prevalence of *Pratylenchus* spp., *Tylenchorhynchus* spp. and *Meloidogyne* spp. was observed on all the three varieties of apple. Variations have also been observed in population ranges of these nematodes. Highest population ranges of *Pratylenchus* spp., *Tylenchorhynchus* spp. and *Meloidogyne* spp. were observed on all the three varieties of apple. In all the apple orchards the simultaneous infestation of two nematode species was 46%, that of three nematode species was 39% and the rest of

15% orchards were found to be infested with four nematode species. The population of each nematode remained more or less the same throughout the year. Maximum numbers were observed during the months of September and October while the populations were the minimum in December and January. *Pratylenchus* spp. was found maximum while *Hoplolaimus* spp. was found minimum throughout the year.

DNA BARCODING: STATUS AND IMPORTANCE IN MEDICAL PARASITOLOGY

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Neglected tropical diseases are affecting over one billion people, which are caused by parasites mostly. Understanding about the epidemiological and ecological attributes of parasitic diseases, monitoring and improved detection can be achieved through correct identification of parasites and vectors. Differentiation of parasites and most vector species based on morphological features is very unreliable. This situation led towards the DNA barcoding implementation, which is a useful technique for the molecular identification of species. In most eukaryotes, mitochondrial cytochrome c oxidase subunit I (COI) gene contains a 650 nucleotides long sequence at 5' end, is the DNA barcode. There are 2.2 million eukaryotes individual sequenced using COI barcode fragment which have been reported in Barcode of Life Data (BOLD) system, 1.3 million being representative species. The most prevalent phyla are Platyhelminthes (15% of species), Nematoda (9%) and Arthropoda (65%). Ectoparasites contribute 35% of species, endoparasites 30% and vectors 33%. There is uneven distribution of about 30775 COI barcodes with length more than 500 nucleotides among these individual out of total and 57% species have no data availability. Mosquitoes are the most sequenced candidates among the arthropod vectors. Endoparasites have fewer number of barcode sequences. The major limitation of DNA barcoding in vectors and parasites is that it is not prioritized in public health or medicine rather funded as biodiversity science. High-volume barcoding is difficult to achieve because of lack of universal primers for the barcode fragment in many taxa. Vector control strategies can be improved through specimen identification. The DNA barcoding along with next generation sequencing (NGS) can detect contamination, heteroplasmy and COI paralogs directly increasing taxonomic confidence.

HISTOPATHOLOGY OF INFECTED LIVER WITH ENCYSTED METACERCARIAE IN SNAKE HEADED FRESH WATER FISH *CHANNA PUNCTATUS* (BLOCH, 1793)

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The tailless encysted late stage or maturing larva of a digenetic trematode parasite that is usually found in an aquatic intermediate host subsequently it always representing the intermediate

host's infectious stage before transfer to its definite host. The present study was carried out to evaluate the histopathological alterations in the liver of fresh water fish *channa punctatus* naturally infected with encysted metacercariae. During the study 95 fresh fishes (*Channa punctatus*) were collected. The collected fishes were dissected and examined for parasites (Encysted metacercariae). Thereafter liver of infected and uninfected liver were retained and fixed in Bouin's fluid for 24 hours. The cysts from infected liver were removed and preserved by using standard techniques of staining and mounting. While, pieces of liver tissues were embedded in paraffin wax and 5-6 μ thick sections prepared. The standard hematoxylin and eosin (H&E) staining techniques were used for histopathological examinations. Microscopic observations of liver sections showed architectural disintegration of hepatocytes, erythrocyte infiltration blood sinusoid, hypertrophy and necrosis of hepatocytes.

**COMPARATIVE EFFICACY OF ORAL FORMULATIONS OF IVERMECTIN
AND LEVAMISOLE UNDER *IN VITRO* CONDITIONS AGAINST
*HAEMONCHUS CONTORTUS***

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Gastrointestinal (GI) parasitism is one of the main constraints limiting the production of livestock population. Different anthelmintics have been used by veterinary practitioners and farmers; however, resistance against anthelmintic drugs remains a major constraint. This study was conducted to estimate the comparative efficacy of oral formulations of levamisole (LEV) and ivermectin (IVM) against *H. contortus* under in vitro conditions. A total of 384 abomasa were purchased for isolation of adult *H. contortus*. The lethal concentration (LC) 90 was calculated using egg hatch test (EHT), larval development test (LDT) and adult motility test (AMT). Probit transformations were performed to transform a typical sigmoid dose-response curve to linear function. A total of 100 eggs/well were incubated in different concentrations of LEV (0.172, 0.086, 0.043, 0.0215, 0.01075 and 0.00538 μ l/ml) and IVM (0.0020, 0.001, 0.0005, 0.00025, 0.000125 and 0.0000625 μ l/ml) to determine the efficacy of these drugs against eggs, larvae and adult *H. contortus*. The LC90 values of both the drugs were found higher than those recommended by WAAVP (0.1 μ g/ml) which indicated development of anthelmintic resistance (AR) against eggs and adults of *H. contortus*. The dose dependent response of LEV and IVM against the adult *H. contortus* also confirmed the resistant worms in the population. Comparative probit analysis of the two drugs indicated significantly higher ($P < 0.05$) efficacy of IVM than LEV. The study provided useful data on the development of AR for recommending an appropriate drug for preventive and therapeutic management of haemonchosis in small ruminant livestock.

**SUSTAINABLE CONTROL OF GASTROINTESTINAL INFECTION THROUGH
INTRODUCTION OF GENETICALLY RESISTANT ANIMALS**

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Small ruminants play a dynamic role in the economy of any country. Their production is very important for their potential to yield meat and milk, while remains a useful source of hair, leather and manure production worldwide. This makes it a good source of income for the rural areas and brings up-gradation of living standards. They can survive and reproduce under high temperatures and low humidity with less availability of feed. Parasitic infections affect small ruminants severely and often reduce their potential to yield for which they are reared. The parasites cause anemia, weight loss, lethargy and diarrhea which results into intensive production losses and ultimately death. Parasitic disease management in sheep goat has been more complex due to emergence of anthelmintic drug resistance strains of parasites. The control of gastrointestinal nematodes (GIN) is mainly based on the use of drugs, grazing management, use of copper oxide wire particles and bioactive forages. Host genetic resistance to parasites, has been increasingly used as a complementary control strategy, along with the conventional intervention methods mentioned above. The strategy of nematode control has evolved to a more practical manipulation of host-parasite equilibrium in grazing systems by implementation of various strategies, in which improvement of genetic resistance of small ruminant should be included. The increasing use of genetic markers (Single Nucleotide Polymorphisms, SNPs) in GWAS (Genome wide association study) or in the use of whole genome sequence data and excess of analytic methods offer the potential to identify loci or regions associated with the nematode resistance. Genomic selection as a genome-wide level method overcomes the need to identify candidate genes. Benefits in genomic selection are now being realized in dairy cattle and sheep under commercial settings in the more advanced countries. Therefore, selection for resistant hosts can be considered as one of the sustainable control strategies. Moreover, it will be more effective when complemented by other control strategies such as grazing management and improving efficiency of anthelmintics currently.

RICKETTSIOSIS: AN EMERGING TICK-BORNE DISEASE IN PAKISTAN

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Rickettsiosis is a disease of public health significance transmitted through different vectors including lice, fleas, ticks and mites. Among these vectors ticks are the most important vectors reported worldwide. A lot of research has been carried out in Europe and Africa and handsome

amount of data is available regarding rickettsiosis. Prevalence of ticks in domesticated animals exceeds upto 50% in different regions of Pakistan including Chakwal, Multan, Layyah, Muzaffargarh etc. Among ticks, *Hyalomma*, *Rhipicephalus*, *Haemaphysalis*, *Amblyomma*, and *Dermacentor* are involved in the transmission of Spotted fever group rickettsia (SFGR) throughout the world. A number of species belongs to these genera are also present in Pakistan e.g. *Hyalomma anatolicum*, *Rhipicephalus microplus*, *Rhipicephalus Sanguineus*. Identification of organism is carried out by using molecular biology in the host as well as in the vectors. Rickettsial species can be detected either from blood of host (Animals and Human) or vector tissue and organs (Midgut and salivary glands) by using molecular tools (qPCR and LAMP) and other serological tests (ELISA and HA, IFA, FAT etc.). In Pakistan very little data is available regarding rickettsiosis. But, the prevalence of vectors is there which needs to be investigated for the presence of rickettsial communities in vectors (Ticks, mites, lice, fleas etc.) and host population e.g. livestock. By knowing the prevalence of rickettsial species in ticks and host, we can devise appropriate preventive measures against rickettsia as well as other vector borne diseases.

TOXOPLASMOSIS: A THREAT TO LIVESTOCK POPULATION

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Toxoplasma is the most successful, model apicomplexan, obligate intra-cellular protozoan parasite affecting both human and animal species. Causes acute to chronic disease known as "Toxoplasmosis". Toxoplasmosis is a disease present worldwide and becoming a global health hazard. In Pakistan toxoplasmosis is present 25% in livestock. Toxoplasma prevalence in Asian countries is 75%. In Bangladesh 4-5 % in Malaysia 7-8%. In Singapore 9-10% in Thailand 11-15% and in Indonesia 17-20%. In India its prevalence is 20%. Toxoplasma can be detected by direct agglutination test, Sabin-Feldman dye test, ELISA and Platelia toxo IGM test. Toxoplasmosis could be congenital or acquired and symptoms include fever, swollen glands, muscle and heart pain and tiredness. Human can acquire infection by consumption of raw or uncooked meat infected with *toxoplasma gondii* cyst, by ingestion of water, soil or vegetables contaminated with oocysts, by blood transfusion or organ transplantation, by trans-placental transmission during pregnancy, drinking raw goat milk. Simple prophylactic measures can reduce the incidence. To control toxoplasmosis we can control the intermediate host and the factors causing the disease. Cook food to high temperature at least 63°C. Freeze meat to below 0°C so reduce chances of infection. Use gloves gardening when contact with soil and sand because it may be contaminated with cat feces containing toxoplasma. Monitoring and surveillance programs and reduce the risk of toxoplasmosis in animals and humans. There is need for more stringent control measures to prevent toxoplasmosis in animals and humans both from disease and economic point of view is emphasized.

STUDIES ON GENUS *HUMBERTIELLA* SAUSSURE, 1869 (MANTODEA : MANTIDAE: LITURGUSINAE) FROM DISTRICT OF MANSEHRA

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During the present study Mantidae fauna of district were collected during the year 2016. A total of 221 specimens of were encountered. Material at hand was sorted out into single genus *Humbertiella* with 02 species i-e: *Humbertiella indica* Saussure, 1869 and *Humbertiella similes* Giglio-Tos, 1917. Additionally, morphological characters along with line drawing are provided.

***GONOCERCELLA MAGNAACETABULUM* N.SP. (TREMATODA: DEROGENIDAE) COLLECTED FROM INTESTINE OF BOWFIN FISH, *AMIA CALVA* (AMIIFORMES: AMIIDAE) FROM RIVER INDUS AT SUKKUR, SINDH, PAKISTAN.**

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During current studies on helminth parasites of freshwater fishes of river Indus, a total of six Bowfin fishes, *Amia calva* (Amiiformes: Amiidae) were collected from the Sukkur and brought to the parasitology laboratory of department of Zoology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan. Host fishes were dissected under stereo dissecting microscope for the examination of gut contents and visceral organs. During examination of these body parts, a total of five specimens of trematodes belonging to genus *Gonocercella* were collected from the intestine of a single host fish. Trematodes were killed in hot water and processed according to procedure given by Garcia and Ash, 1979. Present specimens were compared and differentiated on the basis of rounded anterior as well as posterior end; larger forebody and smaller hindbody; uterus composed of coils; smaller testes, larger ovary situated pre-testicularly overlapping ventral sucker anteriorly and anterior testis posteriorly; vitelline mass large and compact having many lobes. On the basis of these diagnostic differences, a new species *Gonocercella magnaacetabulum* is proposed. The name of new species refers to larger acetabulum and this genus is being reported for the first time from Pakistan.

STUDIES ON PARASITIC PREVALENCE IN WILD TURKEYS (*MELEAGRIS GALLOPAVO*) IN INTENSIVE REARING SYSTEM

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Blood and Fecal samples of wild turkeys (*Meleagris gallopavo*) were collected to analyze parasitic prevalence in turkeys. A total of 800 samples, 400 blood and 400 fecal samples were collected from Avian Conservation and Research Center, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki. Haemoparasites i.e. *Aegyptinella* spp., *Plasmodium* spp. and *Leucocytozoon* spp. were identified from blood samples of *M. gallopavo*. While endoparasites i.e. Nematodes (*Oxyuris mansoni*, *Torameres* spp., *Gongyhmema ingluvicola*, *Ascaridia galli*, *Acuaria hamulosa*, *Ascaridia dissimilis* and *Allodapa suctoria*), Cestodes (*Railliacina* spp. and *Chonnotaenia infundibulum*) and Protozoan (*Eimeria* spp. and *histomonas meleagridis*) were identified in fecal samples of *M. gallopavo*. Tick (*Argas persicus*) Mite (*Cnemidocoptes mutans*, *Ornithonyssus sylviarum* and *Dermanyssus gallinae*) Flea (*Echidnophaga gallinacea*) and lice (*Lipeurus maculosus* and *Amyrsidea perdicis*) were identified as Ectoparasites in turkeys.

NEW RECORD OF GENUS *SUBULURAMOLIN*, 1960 (NEMATODA: SUBULURIDAE) RECOVERED FROM DOMESTIC FOWL *GALLUS DOMESTICUS* OF DISTRICT KHAIRPUR, SINDH, PAKISTAN.

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During current investigation on helminth parasites of Domestic Fowl, a total of 150 hosts were randomly collected from different localities of district Khairpur. Alimentary canal, liver, gallbladder, lungs, kidneys and body cavity were examined under stereodisecting microscope for the presence of nematode parasites. Amongst these hosts examined, 500 specimens (170 ♂ and 330 ♀) of nematodes belonging to genus total specimen of genus *Subulura* Molin, 1960 were recovered from intestine of 60 hosts. Specimens were killed in hot 70% ethanol and preserved in alcohol-glycerol solution. Temporary mounts were prepared in glycerol and lactophenol solutions. Diagrams were made with aid of camera lucida. Specimens were compared with literature and identified as *Subulura* sp. However, this genus is being recorded for the first time from Pakistan.

**INFECTION OF FAMILY ECHINOSTOMATIDAE (TRAMATODA: DIGENEA) IN
PHALACROCORAX NIGER (AVES: PHALACROCORACIDAE) OF SINDH, PAKISTAN.**

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Study was planned to examine infection of helminths generally and family Echinostomatidae particularly in Little Cormorant *Phalacrocorax niger* of district Sanghar, Sindh, Pakistan. Little Cormorants (n=11) were trapped randomly from water bodies of district Sanghar and brought to Parasitology Laboratory of department of Zoology, University of Sindh, Jamshoro. Examination of gut contents and visceral organs revealed four species of family Echinostomatidae *Paryphostomum radiatum* (Duj., 1845) Dietz, 1909; *Paryphostomum sanghari* Abro, 2016; *Petasiger exaeretus* Dietz, 1909 and *Acanthoparyphium* sp. These were mainly collected from intestine of host. Statistically, out of eleven *P. niger* five were infected with *Paryphostomum radiatum* (Duj., 1845) Dietz, 1909 (45.45%), *Paryphostomum sanghari* Abro, 2016 (45.45%) and four were infected with *Petasiger exaeretus* Dietz, 1909 (36.36%) and *Acanthoparyphium* sp. (36.36%). Moreover, overall infection rate of family Echinostomatidae was 45.45 percent.

**PREVALENCE OF HEAD LICE INFESTATION AND ASSOCIATED RISK FACTORS IN
SECONDARY SCHOOL CHILDREN IN LAHORE**

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Head lice infestation (Pediculosis) is a tenacious infection caused by *Pediculus humanus capitis*. A cross sectional study was designed to determine the prevalence of head lice infestations and the risk factors affecting the rate of infestation in secondary school children of Lahore. A total of 399 students of age group 11-16 years were examined, out of which 96 (24.06%) were males and 303 (75.94%) were females. Overall prevalence was 63.16%. Girls were found to be more affected than the males. Visual inspection method by using hand lens was used to detect infestation. The child was considered to be positive for pediculosis if there was any evidence of dead or live nits, nymph or adult lice. The following details were recorded for each child using self-administered questionnaires i.e. history of previous infestation, age, gender, father's occupation, mother's occupation, family income, number of siblings, total rooms in house, hair length and type, hair volume and washing frequency, weekly oiling, regular checking by parents, regular use of fine toothed combs in wet hairs and sharing of accessories among siblings. The chi-square test was applied for analysis. The factors which showed relatively higher prevalence were: 74.58% higher infestation in low age group, 37.5% males and 71.29% females were positive, children with siblings 4 or more were highly infested, siblings sharing a single room showed 91.43% infestation, children with longer hair lengths showed 73.08% infestation, daily hair washing of hair showed the lowest infestation of 42.62%, sharing of accessories (combs, towels, scarves, caps, beddings etc.) showed higher prevalence of 77.17%. It was concluded that the girls were highly infested as compared to boys due to different factors.

A NEW SPECIES OF CESTODE PARASITE IN THE SMALL & LARGE INTESTINE OF RAT (*RATTUS RATTUS* L. 1758) AND MICE (*MUS MUSCULUS* L. 1758) FROM SWAT PAKISTAN

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A new species of the genus *Hymenolepis* (Weinland, 1858) is described here named *Hymenolepis swatensis* sp.n. from the small and large intestine of rat (*Rattus rattus*) and mice (*Mus musculus*). The new species is characterized by having medium sized body, scolex with armed Rostellum. All proglottids are broader than long, testes three in number, Cirrus sac smooth and oblongated. Ovary multilobed. Uterus in form of irregular network; Vitelline gland lobed, parts of uterine wall retained in gravid proglottids. Genital pores Unilateral, Eggs oval and sub-spherical in shape. Embryonic hooks are present with polar filaments.

INDIGENOUS KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) OF PEOPLE ABOUT LEISHMANIASIS AND ITS VECTOR SAND FLY (PHLEBOTOMINAE: PSYCHODIDAE: DIPTERA) LIVING IN AN URBAN SETTLEMENT IN SOUTH PUNJAB, PAKISTAN

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Densely populated cities in the world are facing new health threats vectored by insects and other arthropods with the increase of population. The same is with Multan, a central city of South Punjab in Pakistan, where among many others, cutaneous Leishmaniasis (CL) is being reported regularly. For example, 203 and 58 patients were reported from OPDs of Civil and Nishtar Hospitals, in 2015 and 2016, respectively from Multan. So, the objective of present study is to observe the level of knowledge, attitude, practices, and beliefs of people living in Multan, Pakistan, related to CL. For the study, a cross sectional survey was conducted in Multan city. Total 496 household-respondents were selected by using the Simple Random Sampling (SRS) technique. A semi-structured, pre-tested questionnaire was prepared to collect the data. Multiple choices were given to respondents to collect the information about knowledge, attitude and practices relating to the disease and its vector *i.e.* sand fly. Out of 496 respondents, 73.3 %, 26.2 % and 0.4 % were males, females and she-males, respectively. Respondents had poor knowledge about the vector of the disease as only 2.4 % told that the disease vectored by an insect but no one could tell the name (sand fly). Most of interviewed people (89.1 %) thought that without community participation, the disease cannot be controlled. The results revealed that majority (80.4 %) of target population believed that cleanliness of surroundings helped to save patients from worst condition of the disease. The data so collected would be utilized in management programs against CL and its vector sand fly to save the community from this disease.

STUDY ON HELMINTH PARASITES OF PIGEONS, *COLUMBA LIVIA* GMELIN, 1789 IN CHAKDARA DISTRICT LOWER DIR, KHYBER PAKHTUNKHWA, PAKISTAN

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Present study was aimed to determine the prevalence and taxonomic spectrum of helminth parasites from pigeon hosts. A total of 12 pigeons (including 6 males and 6 females) were collected from different homestead in Chakdara, Lower Dir, KPK, Pakistan. The collected pigeons were brought to the Laboratory of Parasitology, Department of Zoology, University of Malakand for parasite examination. Each of the pigeon was dissected for helminth parasitic infections. Out of the examined, 08 (66.6%) were found to be infected with 4 species of cestodes including *Raillietina* sp. 4 (50%), *Cotugnia* sp. 6 (75%), *Echinococcus* sp. 1 (12.5%) and Pseudophyllidean Cestode 1 (12.5%). Five pigeons, out of the total infected were found to be infected with only one species of parasite, two were found infected with double parasite species while one of the pigeon was reported with three species of parasites in association as: *Cotugnia* sp. + *Raillietina* sp. and *Cotugnia* sp. + Pseudophyllidian Cestode association were reported in one male and one female pigeon, while *Cotugnia* sp. + *Raillietina* sp. + *Echinococcus* sp were noted in one male pigeon. Male pigeons were found more infected than females. In present investigation nematodes, trematodes and acanthocephan components were totally absent. Each of the parasites was discussed with relevant literature and concluded. All the parasites were found in large intestine of the pigeons. During dissection of the pigeons a total of 416 helminth parasites were recovered including *Cotugnia* sp. 373 (89.6%), *Raillietina* sp. 39 (9.37%), *Echinococcus* sp. 3 (0.72%) and Pseudophyllidian Cestode 1 (0.24%) were reported. Each of the parasites recorded herewith photomicrographs, camera lucida drawings and compared with available cestode images. On the basis of the present findings it was concluded that pigeons of the present locality were found infected with cestode parasites, these might be a potential hazardous and injurious not only for the environment but also for humans and other animals in the environment. Such studies should be continuing in future.

SERODIAGNOSIS OF HUMAN TOXOPLASMOSIS IN FAISALABAD, PAKISTAN

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Toxoplasma gondii is an opportunistic parasite that causes high morbidity and mortality in warm-blooded animals including humans. It is a worldwide zoonotic infection usually asymptomatic in healthy individuals but may cause serious complications in immunocompromised hosts. This study was designed to assess the seroprevalence of *Toxoplasma* infection in local population of Faisalabad, to investigate the associated risk factors by the parasite in patients suffering from toxoplasmosis. In the life cycle of *Toxoplasma gondii* intermediate hosts are man and other animals while the final host is cat. All the necessary information was collected with the

help of structurally designed questionnaire and analyzed to find out the risk factors. The blood samples of different age groups from both males and females and children were collected randomly. The serum was analyzed by ELISA to estimate the seroprevalence of toxoplasmosis. Overall prevalence of toxoplasmosis in the local population of Faisalabad was found 57.7%. When gender wise comparison was made, 58.6% males 52% females were found infected by toxoplasmosis. Faisalabad is a city which is expanding at faster rate day by day. This expansion is resulting in the deterioration in the environmental conditions favoring to high incidence of all the parasitic diseases including toxoplasmosis. Public awareness and health education can play a significant role to reduce the incidence of toxoplasmosis in the local population of Faisalabad.

PREVALENCE OF CHEWING LICE (PHTHIRAPTERA: INSECTA) FROM COMMON QUAIL *COTURNIX COTURNIX* (PHASIANIDAE: GALLIFORMES: AVES) FROM JAMSHORO AND HYDERABAD, DISTRICTS, SINDH PAKISTAN

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Host- parasite interaction depends on the environmental and ecological conditions that cause different biological and pathological problems. Chewing lice have a high capability to develop host specificity. In present study the chewing lice (Phthiraptera: Insecta) were examined for their population and rate of infestation on Common quail *Coturnix coturnix* (Phasianidae: Galliformes: Aves) from Jamshoro and Hyderabad Districts, Sindh, Pakistan. The study was conducted during 2013-2014. Twenty six Common Quails were observed in four localities including two urban and two rural areas of Jamshoro and Hyderabad Districts. The selected birds were tagged with identity rings and 5-8 hosts in each locality were examined. Infestation of chewing lice on each bird was checked periodically in each month. Moreover, Permanent Microscopic slides of ectoparasites were prepared through standard method of preservation. During the study, three species of chewing lice were reported from Common Quail. Their population density on host body was recorded in each month. The prevalence of chewing lice species of *Coturnix coturnix* was recorded as 44.47% of *Cuclotogaster cinereus*, 32.64% of *Menacanthus abdominalis* and 22.87% of *Menacanthus cornutus*. The results revealed that high infestation found in birds of rural localities. The present study will help to understand host-parasite interaction in study area.

PREVALENCE OF HELMINTH PARASITES OF SNAKEHEAD MURREL *CHANNA STRIATA* (PERCIFORMES: CHANNIDAE) OF DISTRICT SANGHAR, SINDH, PAKISTAN

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In the present helminthological studies of freshwater fishes, a total of 13 Snakehead Murrel *Channa striata* (Perciformes: Channidae) were collected from District Sanghar, Sindh, Pakistan. Host fishes were examined through gut contents and visceral organs and helminth parasites including acanthocephalan and nematodes were recovered from intestine. The overall prevalence

for these parasites was recorded 61.53%. The maximum prevalence was recorded for acanthocephalans was 65% and for nematodes was 23%. No specimens of trematodes and cestodes were observed during the present helminthological investigation of snakeheaded fishes from Sanghar district.

**BIOMETRY, FEEDING HABITS AND HELMINTH PARASITIC INFECTION IN
EUPHLYCTIS CYANOPHLYCTIS (THE SKITTERING FROG) SCHNEIDER, 1799, IN
DISTRICT, LOWER DIR, KHYBER PAKHTUNKHWA, PAKISTAN**

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The current study aimed to determine the Biometry, food habits and helminth parasites of *E. cyanophlyctis* (the skittering frog) in Chakdara, District, Lower Dir, KPK, Pakistan. A total of 13 frogs were collected, measured, sexes and dissected to assess stomach contents and helminth parasitic infection. The frogs were collected during the period from August to October 2016. Size and weight of the sampled frogs were investigated. The mean body length was, $X=6.08$ cm for females and $x=4.96$ cm for the males. Before evisceration females, mean weight was, $x=28.25$ g and the males, $x=24.4$ g, whereas after this activity, the carcass mean weight was, $x=25$ g in the case of females and $x=21.4$ g in the case male frogs. The mean hind leg length measured, 6.13 cm for female frogs and $x=5.2$ cm in the case male frogs. The hind stylopodium, zeugopodium and autopodium mean length was, $x=1.5$, $x=1.73$ and $x=3.06$ cm respectively in case of female while $x=1.18$, $x=1.72$ and $x=2.5$ cm respectively in case of male frogs. Stomach contents analysis revealed the occurrence of insects, vegetation, and soil particles in all the stomach samples. Among insects, ants, beetles, mosquitoes, wasps, spiders and cockroaches were included. The most frequently consumed prey items among insects have been ants (40/79). They constitute 50.63% of insects. Insects recovered from the stomach contents were identified as belonging to orders, Hymenoptera (ants, wasps), Blattodea (cockroaches), Coleoptera (beetles), Diptera (mosquitoes), and Araneae (spiders). Insects recovered from the stomach contents were compared to those captured from the area. Frogs examined, evidenced for helminth parasitic infection. Four species of helminth parasites were recovered: three species of Nematoda (*Cosmocerca*, *Cosmocercoides* and *Stroglyoides*), and one species of cestode (*Nematotaenia dispar*). *Cosmocerca* was frequently occurring parasite among nematode. Male *E. cyanophlyctis* frogs were smaller than female. They are insectivorous frogs, mostly, infected with nematode parasites.

**FIRST RECORD OF *MENACANTHUS GONOPHAEUS* (BURMEISTER, 1838)
(AMBLYCERA: MENOPONIDAE) FROM HOUSE CROWS IN SINDH PAKISTAN.**

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Crows *Corvus splendens* Vieillot is a wide spread birds in the country that has a variety of ectoparasites as well as endoparasites. The chewing lice of crow here in Sindh have been reported

very less, yet no published data is available so far. During the investigation of crows in different localities of Sindh province including Karachi, Hyderabad, Jamshoro, Sakhar, Larkana, Khairpur, Gambat, Dadu, Mirpur Khas and Thatta, about 200 crows were examined and more than 380 chewing lice have been recovered. This study focused mainly on the first record of crow body louse *Menacanthus gonophaeus* (Burmeister, 1838) (Amblycera: Memoponidae). More than 50 specimens of genus *M. gonophaeus* were being collected; mounted permanently in Canada balsam after passing the standard process of dehydration. The specimens were examined under light microscope and identified as the said species for the first time in Sindh, Pakistan, put a new record for the region.

SOME NEW RECORDS OF DIGENETIC TREMATODES OF EURASIAN COOT *FULICA ATRA* (GRUIFORMES: RALLIDAE) IN PAKISTAN

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In the results of the helminthological survey of Eurasian coot *Fulica atra* (Gruiformes: Rallidae) in Sindh Province of Pakistan, the host birds were collected from various freshwater habitats of Sindh province, Pakistan and examined for the endohelminths. During examination of gut contents and visceral organs on *stereo* microscope, a total of 34 trematodes were collected from intestine, kidneys and lungs of the host birds. These trematodes belong to three genera including *Tanaisia*, *Echinostoma* and *Orchipedum*. Species were identified as *Tanaisia fedtschenkoi* Skrjabin, 1924 with new host and locality record; *Tanaisia longivittellata* Shtrom and Skrjabin, 1947 with new locality record; *Tanaisia atra* (Nezlobinski, 1926) with new locality record; *Echinostoma grande* Bachkirova, 1946 with new locality record and *Orchipedum tracheicola* Braun, 1901 with new host record. Current results are an addition to previously known helminth parasites reported from avian hosts of Pakistan.

NEW SPECIES AND NEW RECORD OF THE GENUS *COLUMBICOLA* EWING, 1929 (PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) FROM *STREPTOPELIA SENEGALENSIS* (COLUMBIFORMES: COULMBIDAE) FROM SINDH, PAKISTAN

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Laughing dove, *Streptopelia senegalensis* (Columbiformes: Columbidae) is a resident bird of Sindh. Laughing doves are found throughout Africa, the Middle East, some parts of Asia and Australia. During the present investigation fifteen birds were examined for their lice from different regions of lower Sindh. The collected specimens of the genus *Columbicola* Ewing, 1929 were mounted permanently in Canada balsam and examined under light microscope for its species identification and morphometry. The specimens were compared with different species of genus *Columbicola* using all related literature and identified as new species. The taxonomic diagnostic differences appeared in head shape and chaetotaxy, male and female terminalia and male genitalia.

The new species has been named in the honour of first authors mother Mrs. Shareefa hence *C. shareefae* has been proposed to accommodate the new record on host and the locality in Sindh, Pakistan.

**NEW RECORD OF A CHEWING LOUSE (PTHIRAPTERA) FROM
COMMON TEAL, *ANAS CRECCA* (ANSERIFORMES: ANATIDAE) FROM
SINDH, PAKISTAN**

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Common teal, *Anas crecca* (Anseriformes: Anatidae) were captured from different water bodies including Hamal Lake and Manchar Lake from Sindh Pakistan. Total six birds were examined for their chewing lice, in which all birds were parasitized by two philopterid species of suborder Ischnocera were collected. These chewing lice were 14 of *Anaticola crassicornis* Scopoli 1793 and four of *Anatoecus icteroides* Nitzsch, 1818, in which the later species has been recorded for the first time on *Anas crecca* from district Larkana and Jamshoro, Sindh, Pakistan, putting the new locality on record. The Specimens were mounted in permanent microscopic slides and were determined with latest literature. The genus *Anatoecus* also reported for the first time from Sindh Pakistan.

**A NEW SPECIES OF GENUS *PARAMONOSTOMUM* LUHE, 1909 (TREMATODA:
NOTOCOTYLIDAE) FROM COMMON POCHARD *AYTHYA FERINA*
(ANSERIFORMES: ANATIDAE) IN SINDH, PAKISTAN**

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During parasitic studies on the migratory birds in Sindh, Pakistan, a total of five Common Pochards (*Aythya ferina*) were captured from the Hamal lake, district Shahdad Kot. During examination of gut contents and visceral organs, seven trematodes belonging to genus *Paramonostomum* Luhe, 1909 were recovered from intestine. Trematodes were processed following Garcia and Ash, 1979. Trematodes were identified and compared with their close allies using latest literature and differentiated on the basis of the body shape and size, position of genital pore, extension of vitellaria, number of uterine loops, shape and position of testes and ovary, post testicular space and egg size. On the basis of these diagnostic differences, a new species *Paramonostomum aythiae* is proposed to accommodate the present trematode. The name of new species refers to the generic name of the type host. However, this genus is being reported for the first time from the bird *Aythya ferina*.

GENUS *PSEUDOMENOPON* MJOBORG, 1910- A NEW GENERIC RECORD OF CHEWING LICE (PHTHIRAPTERA: MENOPONIDAE) FROM EURASIAN COOT, *FULICA ATRA* (GRUIFORMES: RALLIDAE) FROM SINDH PAKISTAN

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Black Coot, *Fulica atra* L. is one of the Palearctic winter visitors which come to Pakistan from European countries in winter during October to March every year. This migratory bird is widely distributed throughout the world. In the present study, black coot has been collected from different water bodies, including Manchar Lake and Hamal Lake from Sindh Pakistan. Seven hosts were examined for their chewing lice; thirteen specimens of genus *Pseudomenopon* Mjoborg, 1910 have been recovered almost all birds. The specimen were dehydrated in graded series of ethanol, cleared in clove oil and permanently mounted in canada balsam; diagram were made through light microscope. Specimens were identified with latest literature and identified as a new species of the genus, however the genus *Pseudomenopon* was also reported as newly recorded from Pakistan. The taxonomic diagnostic differences appeared between head sutures, tergal and pleural plates, male and female terminalia, sub genital plates and male genitalia. On the basis of these taxonomic characters, the new species is proposed with the nomen nudum as *P. sindhiensis* as it was recovered from Sindh region, Pakistan.

***DINNURUS GEDROSIAE* N.SP. IN COMMON DOLPHINFISH *CORYPHYNA HIPPURUS* (PERCIFORMES: CORYPHAENIDAE) OF GAWADAR COAST, BALOCHISTAN, PAKISTAN**

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During current study on metazoan parasites of Common dolphinfish *Coryphaena hippurus* (Perciformes: Coryphaenidae), a total of twelve hosts were collected from the Gawadar coast of Balochistan, Pakistan. All of the twelve hosts examined were infected with *Dinurus gedrosiae* n.sp. Present species differs from its congeners by having pseudosegmentation in posterior half of hind body; oral sucker subterminal; pharynx almost round; esophagus elongated; ventral sucker larger; testes rounded in shape, pre-ovarian, oblique in position, separated from each other; cirrus sac large; ovary rounded, pre-equatorial, submedian, post-testicular, overlapped by uterine loops and vitellaria; uterus densely filled with eggs, starts behind acetabulum reaching up to starting of fourth quarter of hind body; coeca diverticulating at level of ovary, reaching up to posterior half of hind body, encroaching into segmented region of body; excretory tube present; excretory pore terminal; eggs shelled, oval and other varying characters. However, this genus is being reported for the first time from Pakistan.

COMMUNITY BASED MANAGEMENT OF ACUTE MALNUTRITION AND THE PREVALENCE OF THE INTESTINAL PARASITIC INFECTIONS IN THE CHILDREN OF SUJAWAL AND THATTA DISTRICTS OF SINDH, PAKISTAN

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Malnutrition and intestinal parasitic infections are major and common public health and nutrition problems in children in developing countries, caused by the combination of inadequate food intake and intestinal infections. The present study has been aimed to determine and treat the malnourished children as well as the children with the intestinal parasitic infections, and to identify the correlation between stunting, wasting, underweight and intestinal parasitosis. It was aimed to provide the baseline of community based management of acute malnutrition (CMAM) and intestinal parasitic organisms with causes and consequences (focusing on health and hygiene) specifically to the lower Sindh flood-affected districts. The case control study was carried out on 10861 children of age 2 to 14 years of age in village and attending government schools in two flood affected districts of Sindh including Thatta and Sujawal, Pakistan. The study was conducted from March 2013 to April 2015 in which the nutritional assessment was under taken by anthropometry parameters as per WHO standards. Stool samples were collected which were analyzed for the parasitic infections. The prevalence of global acute malnutrition (GAM), severe acute malnutrition (SAM), moderate acute malnutrition (MAM), underweight, stunting, wasting and intestinal parasitosis was reported 18%, 7.9%, 10%, 30.5%, 45.2%, 14.8% and 40.9% respectively. Intestinal parasites were found in all cases of CMAM with SAM 498 (29.4%), MAM 254 (45.6%) and Normal children 3697 (41.5%). Out of 40.9% prevalent result of intestinal parasites, majority (34.9 %) of children result was found in single positive intestinal parasites while as 6% of children were found in multiple parasites. The Parasites encountered during the study were *Ascaris lumbricoides* (4%), *Giardia lamblia* (3%), *Fasciola hepatica* (3%), *Schistosoma mansoni* (3%), Hookworm (2%), *Hymenolepis nana* (2%), *Entamoeba histolytica* (2%), *Trichuris trichiura* (1%), *Taenia spp.* (2%), *Giardia duodenalis* (2%) and 78.5% patients in CMAM got complete cured and were given exit after successful recovery from concerned program. Maternal literacy status, sex and age of the child were significantly associated with malnutrition ($p < 0.05$). Food security and intake data was also calculated which showed that most of the population only eats one time meal in spite of having staple food (Mean= 33.3). Most of the children are not neat and clean while as 57% of the kitchens are located near the cattle garbage areas. It was concluded that the relationship between the prevalence of intestinal parasitic infections and malnutrition among the children of district Thatta and Sujawal showed that 40.9% of the infected children are malnourished (both SAM and MAM). The study confirmed that malnutrition and parasitosis were important child health problems in the region of Thatta and Sujawal.

A NEW SPECIES OF GENUS *CENTRORHYNCHUS* LUHE, 1911 IN JUNGLE BABBLER, *TURDOIDES STRAITUS* (PASSERIFORMES: LEIOTHRICHIDAE) FROM JAMSHORO, SINDH, PAKISTAN

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The study was designed to investigate Acanthocephalan of Jungle babbler *Turdoides straitus* (Passeriformes: Leiotherichidae) of District Jamshoro, Sindh province, Pakistan. A total of 8 birds were captured from study area. During autopsy of gut contents and visceral organs 12 specimens belonging to genus *Centrorhynchus* Luhe, 1911 were recovered from the intestine of hosts. These specimens appeared to be different from its congener in shape and body size, number of longitudinal rows of hooks, number of hooks in each row, size of proboscis receptacle and lemnisci and size of testes. On the basis of major diagnostic differences between present species and its close allies, a new species *Centrorhynchus jamshorensis* n.sp has been proposed. The name of new species refers to name of locality from host birds were collected.

DATA ON CHEWING LICE FAUNA (PHTHIRAPTERA: INSECTA) INFESTING PASSERINE BIRDS OF DISTRICT TANDO ALLAHYAR, SINDH, PAKISTAN

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The chewing lice (Phthiraptera: Amblycera and Ischnocera) of resident birds of order Passeriformes have been reviewed in Tando Allahayar district during 2014 and 2015. During the survey, a variety of passerine birds were trapped randomly from the study site, belonging to 07 families of birds. Birds were examined for their chewing lice through fumigation and visual examination methods. Different species of chewing lice belonging to suborders Amblycera (family Menoponidae) and suborder Ischnocera (family Philopteridae) were collected. These lice were dehydrated in graded alcohol and mounted permanently in canada balsam through standard method. The lice were then examined under light microscope and identified with the help of all related literature. Present study is conducted first time in the region for these ectoparasites of birds. This study revealed 07 species of Menoponidae and 09 species of Philopteridae. The data on chewing lice in the present survey include *Brueelia saleimi* Ansari, 1955, *Colpocephalum fregili* Denny, 1842, *Menacanthus eurysternus* (Burmeister, 1838), *Menacanthus* sp., *Myrsidea splendenticola* Klockenhoff, 1978 and *Philopterus lahorensis* Ansari, 1955 from family Corvidae; *Brueelia channayah* Ansari, 1955, *M. eurysternus* (Burmeister, 1838), *Myrsidea invadens* Kellogg and Chapman, 1902 and *Sturnidoecus sturni* Schrank, 1776 from family Sturnidae; *M. eurysternus* (Burmeister, 1838), *Brueelia* sp. and *Sturnidoecus refractarioles* Zlotorzycska, 1964 from family Passeridae; *M. eurysternus* (Burmeister, 1838) and *Myrsidea pycnonoti* Eichler, 1947 from family Pycnonotidae; *M. eurysternus* (Burmeister, 1838), *Myrsidea sirdianus* Ansari, 1955 and *Philopterus* sp. from family Dicuridae; *Sturnidoecus* sp. from family Emberizidae; and *Brueelia turdoidis* a new species recovered from common babbler (family Turdoididae). All species of chewing lice reported first time from the district Tando Allahyar, Sindh, Pakistan.

**DESCRIPTION OF NEW TREMATODE *PSILOCHASMUS PLATYRHYNCHOSI* N.SP.
(TREMATODE: PSILOCHASMIDAE) IN MALLARD *ANAS PLATYRHYNCHOS*
(ANSERIFORMES: ANATIDAE) OF SINDH, PAKISTAN**

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In result of ongoing helminthological studies of mallard *Anas platyrhynchos* of Kambar Shahdakt District of Sindh Province, Pakistan, a total of 20 birds were captured from different localities. During examination of gut contents and visceral organs, 65 specimens of *Psilochasmus platyrhynchos* n.sp. collected from intestine of the host bird. *Psilochasmus platyrhynchos* n.sp. differs from its close allies in body shape and size, distribution of vitellaria which is densely scattered in hind body, shape of cirrus sac presence of seminal receptacle size and shape of testes and ovary and size of eggs. On the basis of these diagnostic differences, a new species *Psilochasmus platyrhynchos* is proposed.

FIRST RECORD OF GENUS *COSMOCEPHALUS* MOLIN, 1858, IN *PASSER PYRRHONOTUS* BLYTH, 1845 (PASSERIDAE: PASSERIFORMES) FROM SUKKUR, SINDH, PAKISTAN.

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During current studies on the helminth parasites of nematodes of *Passer pyrrhnotus* BLYTH, 1845, total ten nematodes (three ♂ and seven ♀) were collected from the intestine of the four host, killed and preserved in 70% Ethanol and Glycerol solution for detailed study. Measurements are taken in millimeters (mm) and eggs in micrometers (µm). The genus *Cosmocephalus* Molin, 1858 and *Passer pyrrhnotus* is a new host reported for the first time from Pakistan.

THE POPULATION INTENSITY OF TICKS *IXODES* (PARASITIFORMES: IXODIDAE) ON WATER BUFFALO *BUBALUS BUBALIS* (L.) IN VILLAGE CHHAR, TALUKA QAZI AHMED, DISTRICT SHAHEED BENAZIRABAD SINDH, PAKISTAN - A CROSS SECTIONAL STUDY

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Domestic water buffalo *Bubalus bubalis* (Linnaeus, 1758) is a very important economic animal of Sindh Pakistan. Domestic water buffaloes are affected by various types of ectoparasites such as ticks, mites, lice and flies, which cause direct and indirect loss to cattle. Ticks are small arachnids of order Parasitiformes. In the present a cross-sectional study on the prevalence of ticks of family Ixodidae has been encountered and the analysis of usual practices to control these parasites was conducted in village Chhar, taluka Qazi Ahmed, district Shaheed Benazirabad from

September 2016 to November 2016. Survey method was used using geographical map after purposive sampling to select the appropriate locations whereby the ticks can be collected for data collection by mechanical picking method and were then identified in laboratory. A total number of 31 ruminant locations (cattle yards) were visited in the study area in which 214 buffaloes were observed for their ectoparasites. During the study about 4369 ticks were collected, these were observed in large intensity recorded from 19 locations with prevalence of 100%. It was also observed that in 07 locations where fowls were found showed less number of ticks as compared to the 12 locations where there was no fowls found. It was also noticed and analyzed that fowls control the ectoparasites on ruminant domestic livestock and ticks that fall on ground were being eaten by fowls; ticks are mostly prevalent ectoparasites of this area; number of population intensities reduces as the temperature decreases in november. Hence, it was observed that the cultural practices, neat and clean environment and keeping domestic fowls with buffalo are found more effective for the control of ticks subject to the condition of temperature variation.

A NEW SPECIES OF GENUS *STURNIDOECUS* (PHTHIRAPTERA: ISCHNOCERA: PHILOPTERIDAE) ON COMMON MYNA *ACCRIDOTHERES TRISTIS* (PASSERIFORMES: STURNIDAE) FROM HYDERABAD, SINDH, PAKISTAN

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Mynas are most common resident birds in Sindh. Presently it is the first study of chewing lice on mynas in Sindh region. During present study, 12 mynas, *Accridotheres tristis* were examined from Hyderabad, Sindh, Pakistan. *A. tristis* is reported to be infested by three species of genus *Sturnidoecus* in which one species is designated as species novum and other are yet to be identified. Collected specimens of genus *Sturnidoecus* were mounted permanently in canada balsam and studied thoroughly under microscope for their identification, measurement and drawing. The present new specie differs from its congeners in having shape of dorsal anterior head plate, subgenital plate of female, terminal chaetotaxy of male and male genitalia structure. The name of new species of genus *Sturnidoecus* is referred to its type host name, hence *S. tristisae* is proposed.

CYRNEA COLUMBI SP.N. (NEMATODA: SPIRUOIDEA) FIRST AND NEW REPORT OF THE GENUS FROM A NEW HOST *COLUMBIA LIVIA* (PIGEON) IN SINDH, PAKISTAN

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The genus and species *Cyrnea* (Seurat, 1914) *columbi* sp.n. are reported for the first time from a new host *Columba livia* (Domestic Pigeon) in Pakistan. Twelve birds *Columba livia* were

caught from District Nausharo feroze, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. The gizzard of five pigeons were found infected with 130 (08 males, 122 female) specimens. These were fixed in hot steaming 70 % ethanol. Later the nematodes were stored in a solution of 5 parts glycerin and 95 parts 70 % ethanol and cleared in either glycerin or lacto phenol for detailed study. The new species is characterized by having: small, thin worms, dirty or light yellowish in colour. Cuticle with fine transverse striations. Mouth terminal, with two well developed lateral lips. Each labia possess pair of papillae. Vestibule long, roughly U shaped provided with less chitinized walls. Esophagus long, divided into short muscular and long glandular portions. Male smaller and thinner than female. Tail wings well developed, asymmetrical, contains 6-7 pairs of stalked papillae, of which 4-5 pairs are pre-anal, one pair ad-anal and two pairs are post-anal. Two to three large lateral papilla are present in male specimens at posterior region of the body. Spicules are an equal and dis-similar in shape. Smaller spicule having simple proximal end while the longer spicule has typical proximal end with three unequal dissimilar processes. Gubernaculum irregular in shape, situated at distal end of the spicule. Female larger than male specimens. Vulva lies in the posterior region. Tail is rounded in shape. Eggs double walled. The present specimens do not match with the reported species of the genus *Cyrnea* and thus regarded as a new species. The species name refers to the host (*Columba livia*).

***CENTRORHYNCHUS CRIBBI* SP. N. (ACANTHOCEPHALA: CENTRORHYNCHIDAE)
IN THE *CENTROPUS SINENSIS* (PHEASANT CROW) FROM NAUSHARO FEROZE,
SINDH, PAKISTAN**

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Centropus sinensis is an omnivorous bird, feeding on wide range of insects, caterpillars, saw scaled vipers. They also eat bird eggs, nestling, fruits and seeds. Ten *Centropus sinensis* (Pheasant crow) were collected, from Nausharo feroze District at random intervals and brought to the parasitology laboratory, Department of Zoology, University of Sindh, Jamshoro, Pakistan. The birds were anaesthetized, autopsied and examined for presence of helminth parasite infection. The worms were mounted permanently according to standard procedure. 60 acanthocephalans (56 males and 4 females) were collected from the intestine of *Centropus sinensis*. These belong to the genus *Centrorhynchus* Luhe, 1911 and a new species *Centrorhynchus cribbi* sp.n. is proposed. The new species is characterized by having: Male body elongated much smaller as compared to females, slender, slightly curved in some specimens with antero-dorsal hump. Proboscis small, globular not divided into anterior and posterior parts. Proboscis with 14-16 rows of hooks, each row has 12-14 hooks. Anterior hooks simple and long, while the posterior are gradually smaller. Neck short broader as compared to longer. A large number of nuclei are present in the trunk region of varying sizes. Proboscis receptacle double walled, elongated inserted at the middle of the proboscis with a cephalic ganglion. Lemnisci two, long, slightly sub equal with many small nuclei. Testes in the anterior half of the body. Cement glands are four. Elongated, tubular. Saeffigen's pouch prominent, elongate. Bursa well developed in most specimens protruded out. Female Body elongated slightly

curved in a few specimens with antero-dorsal hump. The hooks arrangement and size are similar to male specimens. Gonospore sub-terminal, uterus long, vagina complex. Eggs oval, numerous, without polar prolongation of middle shell, starting from proboscis receptacle reaching almost to the posterior end, filling most of the body.

***HETEROTESTOPHYES GIBSONI* SP.N. (TREMATODA:HETEROPHYIDAE) FROM THE BIRD *STERNULA ALBIFRONS* (LITTLE TERN) IN SINDH, PAKISTAN.**

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In result of ongoing helminthological studies on *Sternula albifrons* (Little tern) were shot down from the District Jamshoro, Sindh, Pakistan and brought to the Parasitology laboratory of the department of Zoology, University of Sindh, Jamshoro. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. The specimens were collected from the small intestine of *Sternula albifrons* and mounted permanently according to standard procedure. A detail study was conducted and identified as belonging to genus *Heterotestophyes* Leonov, 1957 and is proposed *Heterotestophyes gibsoni* sp.n.. The new species is characterized by having: Body small, divided into fore and hind body. Oral sucker terminal, rounded. Ventral sucker muscular, rounded, occupy 2nd quarter of the body, closer to anterior extremity. Testes lie in posterior most region of the hind body, these are Juxta-opposite, rounded in shape. Genital atrium occupies middle of the body, elongated to rounded in shape, Spines in the crown are 57-60 in number and genital opening inside the genital sac, above the spines. Seminal vesicle clearly not obvious due to profuse uterus. Ovary pre-testicular, rounded in shape. Uterus profuse with loops in hind body, Vitellaria follicular, commence at little distance below the genital atrium, extend up to the hind body above the testes, Eggs are oval shaped, double walled. The Species name refers to Dr. D.I. Gibson, Former head of Parasitology section, British museum, London.

***HETEROTESTOPHYES HECKMANNI* SP.N. (TREMATODA:HETEROPHYIDAE) FROM THE BIRD *STERNULA ALBIFRONS* (LITTLE TERN) IN SINDH, PAKISTAN.**

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Parasitic infection is a serious health problem in the world, especially in developing countries. This study was conducted to find the Helminth parasites in Little tern (*Sternula*

albiferons) from District Jamshoro, Sindh, Pakistan. Fifteen birds were dissected, out of fifteen, ten were found infected. A total 20 trematodes were collected from the intestine of Little terns. The trematodes were fixed, preserved, dehydrated, stained and finally permanently mounted in Canada balsam for further detailed study and identified as belonging to the family Heterophyidae Leiper, 1909, genus *Heterotestophyes* Leonov, 1957 and proposed as *Heterotestophyes heckmanni* sp.n. Comparatively few species of the genus are reported from the avian hosts throughout the world. The new species differ from already reported species of the genus *Heterotestophyes* in having different size and shape of the body, oral sucker without transverse ridge, short pre-pharynx, large pharynx and esophagus, position and size of acetabulum, size of genital sac, crown of spines, shape and size of gonads, arrangement of vitelline follicles. *H. heckmanni* sp.n. is a first record of the genus from a new locality Jamshoro, Sindh in Pakistan. The new species refers to Dr. Richard A. Heckman, well known and famous emirates Parasitologist in USA.

SERO-COPROLOGICAL ELISA DETECTION OF LIVER FLUKES (*FASCIOLA HEPATICA*) IN DIFFERENT GOAT BREEDS OF MULTAN, PAKISTAN

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The purpose of this study was the early detection of *Fasciola hepatica* in goats due to difficulty in early detection of this disease (Liver Fluke). The study was conducted randomly on goat (*Capra hirsca*). About 100 samples were collected from local breeds of goat in and around Multan region. Serum was collected and separated as per procedure and stored at 4°C for further use. ELISA kit was purchased from Biox, Belgium, for sero-coprolological diagnosis of *Fasciola hepatica* in feces and serum of the host(s). The fecal samples of animal(s) was examined by various parasitological examination methods including Sedimentation Method, Flotation Method and by light microscopic methods for further confirmation. The copro-antigen ELISA results were collected and analyzed statistically by mean and SD and OD value. The Overall prevalence was recorded as 33%, 25% and 19% respectively. It was observed that the copro-antigen ELISA method was highly significant than other routine methods.

MOLECULAR DETECTION AND PREVALENCE OF *HEPATOZOON CANIS* IN DOGS FROM PUNJAB (PAKISTAN) AND HEMATOLOGICAL PROFILE OF INFECTED DOGS

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The intraleukocytic parasite, *Hepatozoon canis*, causes the sometimes fatal tick borne disease canine hepatozoonosis. In this study, dogs from Islamabad, Lahore and Multan Districts of the Punjab region of Pakistan were surveyed to investigate the presence and prevalence of *H. canis* infection and to determine the effects of the parasite on hematological parameters. Blood samples were collected

from 151 domestic dogs (149 pet, 2 stray) of both sexes and varying ages. Data on sex, age, tick infestation and clinical factors (body temperature, mucous membrane status, and presence of haematuria and vomiting) were collected. Using PCR, 18 dogs (11.9%) were found positive for the presence of *H. canis* DNA. Partial sequences of the 18S rRNA gene shared 99–100% similarity with the corresponding *H. canis* isolates. This epidemiological survey revealed higher prevalence of *H. canis* in Islamabad (11/49, 22.4%) compared to Lahore (3/52, 5.8%) and Multan (4/50, 8%) in Pakistan. No investigated epidemiological or clinical factors was found to be associated with the presence of *H. canis* ($P > 0.05$) in dogs. *Hepatozoon canis* positive dogs exhibited higher minimum inhibitory dilution ($P = 0.04$), mixed inclusion ($P = 0.008$) and relative distribution width of red blood cells ($P = 0.02$) and lower haematocrit ($P = 0.03$) and mean haemoglobin content ($P = 0.03$) than did dogs in which *H. canis* was not detected. We are recommended this PCR based protocol to the veterinary practitioners for the detection and/or confirmation of *H. canis* in dogs suspected for hepatozoonosis to improve their health status.

PREVALENCE OF PARASITIC-INFESTATION IN DOMESTIC PIGEONS AT MALAKAND REGION, KHYBER PAKHTUNKHWA, PAKISTAN

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As there is no data available on parasitic infestation on pigeons in and around Malakand division, the present study was therefore designed to determine the prevalence of ecto- and endo-parasites of domestic pigeons at Malakand region, KPK, Pakistan. A total of 15 adult pigeons (10 males and 5 females) were examined. Out of the total pigeons examined, 13 were found to be infected with three species of ecto-parasites and 10 pigeons were found to be infected with three species of endo-parasites. The three species of ecto-parasites identified were lice of the genera *Columbicola columbae* 86.6% (n=13/15), *Campanulotes bidentatus* 46.6% (n=7/15) and *Menacanthus stramineus* 33.3% (n=5/15) with overall prevalence of 86.66%. No ticks and mites were recorded. Single ecto-parasitic infection (40%) was more prevalent as compared to double infection (13.33%) and triple infection (33.33%). The three species of endo-parasites identified were two species of cestodes includes *Raillietina* spp. 60% (n=9/15) and *Cotugnia* spp. 13.3% (n=2/15). Only one nematode *Ascaridia* spp. constituted the lowest prevalence of 1(6.66%) was reported. No trematodes and acanthocephalan were reported. No significant difference ($P=0.0194$ for lice and $P= 0.5166$ for endo parasites) was found between examined and infected of male and female pigeons. The pigeons had higher prevalence of single endo-parasitic infection (53.33%) as compared to double infection (33.33%). As parasites are responsible for transmission of various infectious agents and are also responsible for various clinical and sub-clinical diseases we recommend further studies in assessing the prevalence of pigeon's parasitic infection and effect of parasites on pigeon's health and production to assist the clinicians regarding epidemiological forecasting and aware the farmers to take appropriate measures against them.

**CYRNEA COLUMBI SP.N. (NEMATODA: SPIRULOIDEA) FRIST AND NEW REPORT
OF THE GENUS FROM A NEW HOST COLUMBIA LIVIA
(PIGEON) IN SINDH**

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The genus and species *Cyrnea* (Seurat, 1914) *columbi* sp.n. are reported for the first time from a new host *Columba livia* (Domestic Pigeon) in Pakistan. Twelve birds *Columba livia* were caught from District Nausharo Feroze, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. The gizzard of five pigeons were found infected with 130 (08 males, 22 females) specimens. These were fixed in hot steaming 70% ethanol and cleared in either glycerine or lacto phenol for detailed study. The new species is characterized by having; small, thin worms, dirty or light yellowish in colour. Cuticle with fine transverse striations. Mouth terminal, with two well developed lateral lips. Each labia possess pair of papillae. Vestibule long, roughly U shaped provided with less chitinized walls. Oesophagus long, divided into short muscular and long glandular portions. Male smaller and thinner than female. Tail wings well developed, asymmetrical, contains 6-7 pairs of stalked papillae, of which 4-5 pairs are pre-anal, one pair ad-anal and two pairs are post-anal. Two to three large lateral papilla are present in male specimens at posterior region of the body. Spicules are un equal and dis-similar in shape. Smaller spicule having simple proximal end while the longer spicule has typical proximal end with three unequal dissimilar processes. Gubernaculum irregular in shape, situated at distal end of the spicule. Female larger than male specimens. Vulva lies in the posterior region. Tail is rounded in shape. Eggs double walled. The present specimens do not match with the reported species of the genus *Cyrnea* and thus regarded as a new species. The species name refers to the host (*Columba livia*).

SECTION - V**FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER
BIOLOGY, MARINE BIOLOGY****1. ECOLOGY AND ENVIRONMENTAL POLLUTION****POPULATION ESTIMATION AND THREATS TO INDIAN HARE (*LEPUS
NIGRICOLLIS*) IN RAKH-SARDARAN GAME RESERVE, DISTRICT HARIPUR**

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A study on Population Estimation and threats to Indian Hare in Rakh Sardaran Game Reserve District Haripur was conducted during September 2015 to May 2016. A total of 98 Indian hare were observed in 15 visits. Reconnaissance survey in the habitat of Indian hare was done and 5 potential sites were selected for further study. Direct and indirect method was used to observe Indian hare in Rakh Sardaran Game Reaserve, Line transect method was used to determine population density of Indian hare. One transect of different length was run in each side site. Population density of Indian hare was recorded as 6.533/ Km². Male ratio (63) was higher as compare to females (35). Population density of Indian hare was observed maximum at Chamba pend (30) due to less human disturbance, good quality cover and vegetation. Least population density was observed in Morhi pir bakhsh (13). Threats to population of Indian hare were analyzed by constructing questionnaire which was filled by Wildlife staff and local community. The maximum threats to population and habitat were deforestation and Poaching least was recorded as forest fire.

**BIOLOGICAL AFFINITIES AND VARIATIONS OF THE KOHISTANI POPULATION
INTO THE WORLD PERSPECTIVE**SAWAR KHAN,^{1,3*} HABIB AHMAD,² MIAN SAYED KHAN,¹
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The northern of Pakistan holds a unique position on the geographic, historic, cultural and ethnic maps of the world. The Kohistan District lies at the knot of spectacular mountain ranges the Himalayas, the Karakorum and the Hindu Kush. This study was aimed to analyse the biological affinities and variations among the Kohistanis into the world perspective. Two standard approaches i.e., dental nonmetric traits analysis and mtDNA analysis were used to accomplished the task.

Dental impressions and oral swab were collected from the Kohistani people. Dental nonmetric trait frequencies were scored according to ASUDAS. The Kohistani dental morphology profile into the world perspective showed affinities to Western Europeans, Modern Indians and Indo-Iranians while it was much divergent of Chinese-Mongolians, Southern Siberians and Southeast Asian. Total genomic DNA was isolated, HVS1 of mtDNA was amplified and sequenced for haplotyping. The sequences were aligned against rCRS and variations were observed. The Kohistani mtDNA haplotypes were found to belonging 12 different haplogroups. The predicted haplogroups with their frequencies were: H14a (11.76%), U5b (5.88%), U7 (17.64%), N1a (5.88%), M33b (5.88%), M31a (5.88%), W4a (5.88%), N (5.88%), H1a (5.88%), D4g (5.88%), R5a (17.64%) and H14b (5.88%). The predicted haplogroups showed the same patterns of genetic affinities for Kohistani population into the world perspective as were obtained from dental morphology analysis.

**RISK ASSESSMENT AND MITIGATIVE MEASURES,
TOWARDS SUSTAINABLE ENVIRONMENTAL IN A SMALL TOWN OF
PUNJAB, PAKISTAN**

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Landfills have been increasing in the recent years with little to no effort being made to contain the situation. The main issues are of improper garbage disposal and poor sewerage system both of which amplify the severity of one another. As solid waste is increasing all over the world, the town of Rabwah has also seen a rapid increase in landfills, where there are about 100 landfills with 3-4 places being added per year since. Only 75 of these places are cleaned after 15-20 days on average while the rest of the 25 never cleared. Multiple efforts were made to make the public aware of the situation by educational institutions and city organizations. Suggestions of plans and solutions were given to the authorities to overcome the morbidity imposed on environment by the rapid increase in the magnitude of the stated problems. The methodology used included surveys of the city, seminars, reuse exhibitions, 3R festival, playing of videos in educational institutions, brochures and booklets distribution and display of banners. Authorities were suggested to construct more public dustbins equidistant to each other so that the disposal of garbage at improper locations becomes less frequent. Moreover the clearance of these dustbins more frequently by professional management was also stressed upon. The public was instructed to follow 3-R principles because of being the most sustainable, economic and best option towards a better world. As a result of three years efforts mindsets of residents has changed, several places have since been cleared. The use of polystyrene cups has drastically decreased from 72,000 to 0 per annum in one institute and the author has become a member of the Asian Pacific 3R forum. At this rate, it would only take 5-6 more years before we see a drastic impact of these efforts in Rabwah town.

2. FRESHWATER BIOLOGY AND FISHERIES

STUDY ON DIVERSITY AND DISTRIBUTION OF GENERA *SCHIZOTHORAX* AND *SCHIZOTHORICHTHYES* IN RIVER SWAT

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The present study was conducted to explore the diversity and distribution of two genera *Schizothorax* and *Schizothorichthys* in the river Swat in 2015 in twelve locations from Madyan to Mingora by weekly collection using Cast net, Mesh net, Hooks and Fishing rods. A total of 28 specimens, including 6 species belonging to genera *Schizothorax* and *Schizothorichthys* were investigated. The specimens were identified based on their morphometric and meristic counts and fin formula. The species found in river Swat were *Schizothorax plagiostomus*, *Schizothorichthys esocinus*, *Schizothorichthys labiatus*, *Schizothorax richardsonii*, *Schizothorax sinuatus* and *Schizothorichthys macrophthalmus*. Among these species, *Schizothorax richardsonii* is considered as vulnerable and is a valuable game fish species according to IUCN 2017 and needs urgent protection and proactive conservation efforts to save from becoming extinct in most of its range. The most abundant species of river swat was *Schizothorax plagiostomus* while the least abundant species was *Schizothorichthys macrophthalmus*. However, all of the species of genera *Schizothorax* and *Schizothorichthys* facing drastic decline in their distribution range and within river Swat due to introduction of exotic salmon and overfishing.

EFFECT OF CORN BASED FEED INGREDIENTS WITH VARYING PROTEIN LEVELS ON GROWTH PERFORMANCE OF *LABEO ROHITA*

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A feeding trial was carried out for 120 days to observe the effect of corn as a carbohydrate source both in gelatinized and non-gelatinized form at different protein levels i.e. 30%, 35% and 40% on performance of growth and morphometric characteristics in *Labeo rohita*. Fingerlings were distributed randomly to aquaria of each having dimensions 90L×30W×45H (cm) with 29 L water capacity. Two replicate were used per treatment with the stocking of fifty fingerlings in each replica. Six test diets were prepared i.e. T₁: G, 30% CP; T₂: NG, 30% CP; T₃: G, 35% CP; T₄: NG, 35% CP; T₅: G, 40% CP and T₆: NG, 40% CP. After acclimatization of one week, fingerlings were fed with test diets twice daily to satiation at 4% of live wet body weight and the fingerlings were transferred to other aquaria fortnightly for weight and length measurement. At the termination of experiment, highest average body weight gain and total length was exhibited by T₅ (G, 40% CP) which were statistically non-significant. These findings concluded that gelatinized corn at 40% protein level proved as a promising fish feed ingredient being more efficiently utilized and showed better growth results in *Labeo rohita*.

APPRAISAL OF WATER QUALITY PARAMETERS AND HEAVY METAL IN WATER, SEDIMENTS AND FISH MAHSHEER (*TOR PUTITORA*) OF RIVER POONCH AT DISTRICT KOTLI AZAD JAMMU AND KASHMIR (AJ&K), PAKISTAN

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The current study were inspected with the study of selected quality parameters of water and heavy metal contents of water, sediments and fish in river Poonch at district Kotli Azad Jammu & Kashmir (AJK). Samples having sediments, water and fish were gathered including six different sites in the period of April to June 2015. During the study period the mean value recorded for water quality parameters were: pH 9.20, mvORP 32.2, DO 6.95mg/l, EC 252.96 μ S/cm and 271.91 μ g/cm^A, TDS 197.03, Salinity 0.77, Turbidity 3.02, Temp 31.65 and Pressure 13.37. Mean values recorded for heavy metal absorption in water, sediments and fish were below the maximum permissible levels. The current study aims was to show that this water is good for the existence of fish survival and growth. So this study exhibits that the part of Poonch River at district Kotli having water quality parameters and heavy metals were within the tolerable range and no harmful effects on the fish growth and reproduction.

WATER QUALITY ASSESSMENT OF TATTA PANI (THERMAL SPRING) KOTLI AZAD JAMMU AND KASHMIR

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Water of thermal spring (Tatta Pani) and adjacent spring was studied for its Physico-chemical pollution and biological (E.coli) load on monthly basis from July, 2015 to February, 2016. The physical parameters such as color of water, odor (Rotten eggs) of water, air temperature (21.32 \pm 1.40 to 26.43 \pm 2.34 °C) and water temperature (61.12 \pm 0.170 to 18.88 \pm 1.28 °C) were recorded on the spot. The chemical parameters of water were also analysed in the laboratory and found that the mean value of pH ranged from 6.72 \pm 0.03 to 7.58 \pm 0.11), turbidity (2.262 \pm 0.31 NTU to 2.562 \pm 0.46 NTU), calcium ions (56.08 ppm \pm 1.85 to 142.2 ppm \pm 7.38), magnesium ions (75.6 ppm \pm 4.29 to 105.6 ppm \pm 6.54), total alkalinity (4.42 ppm \pm 0.312 to 8.27 ppm \pm 1.14), chloride ions (2.34 ppm \pm 0.176 to 8.85 ppm \pm 0.47), sulphide ions (323.3 ppm \pm 14.18 to 439.6 ppm \pm 26.04), total suspended solids (200 \pm 53.45 mg/L to 350 \pm 62.67 mg/L), total dissolved solids (562.5 \pm 113.2 mg/L to 937.5 \pm 113.2 mg/L), total organic solids (375 \pm 125 mg/L to 937.5 \pm 305.2 mg/L), total inorganic solids (500 \pm 0 mg/L to 625 \pm 81.83 mg/L respectively). Thermal spring (Tatta Pani) was completely lack the flora and fauna and E.coli, however they were present in adjacent springs, particularly in summer season owing to heavy rains.

**MORPHOMETRIC STUDIES OF *GLYPTOTHORAX KASHMIRENSIS*
(KASHMIR CATFISH)**NAGINA GILANI¹, MUHAMMAD RAFIQUE² AND SHAMIM AKHTER*¹¹Department of Zoology, PMAS Arid Agriculture University, Rawalpindi²Pakistan Museum of Natural History, Islamabad

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Glyptothorax kashmirensis (Kashmir catfish), an endemic fresh water fish species has been reported from different parts of Pakistan and Azad Kashmir. Due to continuous decline of population of *Glyptothorax kashmirensis*, it has been declared as critically endangered by the IUCN, 2014. Morphometric study is the first and most important step towards development of management and conservation strategies that determines not only the differences in an individual population but also discriminates different species in a genus. In present study, 19 variants of this species were collected from River Jhelum and Poonch (Azad Kashmir) and River Kurram (KPK). A total of 33 parameters were studied for all the variants collected including color, size/shapes of morphological characters, meristic counts and body measurements (head, eyes, fins, barbells, adhesive organ and cleft of mouth). Body measurements were taken using vernier caliper with 0.05mm accuracy and sucker pleats and fin rays were observed using magnifying glass and light microscope. Most distinguishing feature of *Glyptothorax kashmirensis* was the thoracic adhesive apparatus with length slightly larger than width having oval or rounded shape with a clear depression in the center. Body was elongate with dark greyish color on dorsal and light yellowish color on ventral side. Skin was somewhat granular but without scales. Head was slightly depressed and longer than broad. Eyes were small and rounded in shape. Four pairs of barbels were observed with nasal barbels smaller than inner mandibular barbels. Maxillary barbels were reaching beyond occipital process and outer mandibular barbels reaching beyond the beginning of pectoral fin; this feature also distinguished *Glyptothorax kashmirensis* from other species of genus *Glyptothorax*. Total body length of populations studied ranged from 82mm-152mm and maximum body depth was 13mm-37mm at the base of dorsal fin. Serrations were present on the dorsal, pectoral and pelvic fin. Caudal fin was forked with lower lobe larger than the upper one. The morphometric data on Kashmir catfish will not only be useful for defining its taxonomic status but will also help while designing and implementing conservation strategies.

**EFFECT OF REPLACEMENT OF FISH OIL BY CANOLA OIL ON GROWTH
PERFORMANCE, FATTY ACID DIGESTIBILITY AND MEAT QUALITY
OF *LABEO ROHITA* FINGERLINGS**SIDRA ALTAF, MUHAMMAD AFZAL, SYED ZAKIR HUSSAIN SHAH* AND
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Present research work was planned to determine the effect of replacement of fish oil by canola oil on growth performance, fatty acid digestibility and meat quality of *Labeo rohita* fingerlings. Four experimental diets were formulated with 9% lipid originating from fish oil which was substituted in 33% increment by canola oil. Water quality parameters i.e. temperature,

dissolved oxygen and pH were monitored throughout the feeding trial. Each experimental diet was fed in duplicate, twice a day to apparent satiation for 60 days. Fecal matter was collected daily for determination of fatty acid digestibility. Growth and survival rate were observed every week during the whole experimental period. At the end of the trial, from each replicate, fingerlings were collected to analyze the muscle proximate composition. Data were subjected to one-way analysis of variance (ANOVA) under completely randomized design (CRD). Replacement of fish oil with canola oil showed similar results for the growth performance of *L. rohita* fingerlings as was observed from fish oil up to the level of 33%. Replacement of fish oil with canola oil also showed variations in the digestibility of saturated, unsaturated and polyunsaturated fatty acids. Crude protein content in muscles remained unaffected by the replacement of fish oil with canola oil while crude fat content significantly increased with increase in the replacement level. In conclusion, present study evidenced that fish oil can be replaced by canola oil up to the level of 33% without affecting the growth performance, but the crude fat content in the muscles of fingerlings significantly increased with increase in the replacement level. Moreover, replacement of fish oil with canola oil showed variations in the digestibility of fatty acids in *Labeo rohita* fingerlings.

**INFLUENCE OF DIETARY LIPID LEVELS AND VITAMIN E ON GROWTH
PERFORMANCE, LIPID PEROXIDATION AND FATTY ACID PROFILE OF
LABEO ROHITA FINGERLINGS**

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The effect of dietary lipids and vitamin E supplementation on growth performance, lipid peroxidation and fatty acid profile of liver of *Labeo rohita* fingerlings was experimentally evaluated in a present 2×3 factorial experiment. Lipid peroxidation was determined in terms of α -tocopherol level, thiobarbituric acid reactive substances (TBARS) and antioxidant enzyme activities. Six experimental diets were made by supplementing two levels of lipids (8 and 16%) and three levels of vitamin E (0, 100, 1000 mg/kg). At the onset of feeding trial, twenty-five fish were stocked randomly into triplicates for each dietary treatment with near uniform biomass (initial body weight 3.33 ± 0.03 g). Weight gain was measured fortnightly for determination of the growth performance. At the end of 60 days feeding trial, fish were sacrificed and liver samples were collected and analyzed. Fish fed the 16% lipid diets showed significantly higher weight gain and specific growth rate than fish fed the 8% lipid diets. Similar significant increase in these growth parameters was also observed as a result of vitamin E supplementation and interaction of both the supplements. Supplementation of lipids, vitamin E and interaction of both supplements improved feed conversion ratio and protein efficiency ratio. Increasing the dietary supplemental levels of fish oil from 8 to 16% significantly decreased the liver α -tocopherol content. Liver α -tocopherol contents were also significantly increased with increment of dietary vitamin E. Liver TBARS and antioxidant enzyme (superoxide dismutase, catalase and peroxidase) activities increased with increasing dietary fat levels but decreased with increasing vitamin E supplementation. Liver saturates and n-6 polyunsaturated fatty acids (PUFA) decreased significantly, whereas monounsaturated fatty acids (MUFA) increased with increment of dietary fish oil. Dietary levels of

vitamin E significantly increased DPA and DHA (n-3 PUFA) while decreased palmitic acid and stearic acid (saturated fatty acids) in liver of *L. rohita*. In conclusion, increased lipid levels enhance the growth performance of *L. rohita*, however, increased the rate of lipid peroxidation, to cope with which, increased level of dietary vitamin E is required.

EFFECT OF DIETARY ACIDIFICATION ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN SILVER CARP, *HYPOPHthalmichthys molitrix*

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Present research work was planned to evaluate the effect of dietary acidification on nutrient digestibility and growth performance in *Hypophthalmichthys molitrix* fingerlings. Five experimental diets were prepared and designated as T1, T2, T3, T4 and T5. The experimental diet T1 served as control diet while T2, T3, T4 and T5 were contained 2% of malic acid, citric acid, formic acid and lactic acid, respectively. These diets were fed to fish once a day near satiation level for 12 weeks. Chromic oxide was added at 1% concentration in experimental diets as inert marker for nutrient digestibility estimation. Fecal material was collected throughout the feeding trial for digestibility analysis. Water quality parameters were kept constant throughout the experiment. Dietary supplementation of organic acids showed significant improvement in growth performance. Maximum increase in growth performance was observed by feeding citric acid added diet. Inclusion of all organic acids showed significant effect on the digestibility of dry matter, crude protein and crude fat while non-significant effect was observed on ash digestibility, when compared to control. Highest mineral digestibility was observed in the diet supplemented with citric acid. Except Na, digestibility of P, Mg, Ca, K, Zn, Fe, Cu and Mn was significantly improved ($p < 0.05$) in fish fed soybean meal based diet supplemented with organic acids (malic acid, citric acid, formic acid and lactic acid) compared to control. Conclusively, this study indicates that addition of 2% organic acids in soybean meal based diets improved the growth performance and nutrient digestibility performance of *H. molitrix* fingerlings.

ESTIMATION OF TRACE MINERAL RETENTION BY ACIDIFICATION AND PHYTASE SUPPLEMENTATION IN A MAJOR CARP

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The aim of present study was to estimate the effects of dietary microbial phytase and citric acid supplementation in improvement of trace mineral retention in *Labeo rohita*. Three test diets were prepared having 2% citric acid, 1500FTU phytase and third was having both 3% citric acid and phytase, with one control diet having no acidification and phytase addition. After the

compilation of data it was found that acidification and phytase supplementation significantly affected the trace mineral retention in fish and both synergistically enhanced the retention of minerals in major carp (*Labeo rohita*).

HAEMATOLOGICAL RESPONSE OF *OREOCHROMIS NILOTICUS* TO ACUTE AND CHRONIC Pb+Cd METAL MIXTURE EXPOSURE

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From the last few years, aquatic ecosystems are contaminated with effluents released from various sources due to which aquatic life has been disturbed. The objective of present study is to determine the effect of acute and chronic Pb+Cd metal mixture on blood cells of *Oreochromis niloticus*. For this purpose, fish fingerlings were purchased from Fish Seed Hatchery, Faisalabad. For acclimatization, fingerling were kept in cemented tanks. After acclimatization, experimental trial was conducted by keeping fish fingerlings in acute and chronic Pb+Cd metal mixture stressed conditions. Three aquaria were selected as control, acute and chronic with three replicates. The physico-chemical parameters viz. dissolved oxygen, water temperature, total hardness, electrical conductivity and pH were analyzed during the whole experimental trial. Each aquarium was stocked with 10 fishes. Duration of the experimental trial was remained for 2 weeks. At the end of experimental trial, blood samples were collected in EDTA tubes from experimental and controlled group and various hematological parameter such as RBC, PLT, WBC, MCV, HGB, PCT, PDW, MCHC, MCH, and HCT were measured by following standard methods. Selected blood parameters value were found as WBCs $145.1 \times 10^3 \mu\text{L}^{-1}$, RBCs $0.2 \times 10^3 \mu\text{L}^{-1}$, HGB 0.6gdL^{-1} , HCT 3.4%, MCV 163.2fL, MCH 29.6pg, MCHC 10.2gdL^{-1} , PLT $25 \times 10^3 \mu\text{L}^{-1}$, PCT 0.02%, and PDW 8.2fL due to acute Pb+Cd metal mixture stressed *O. niloticus*. Whereas value of WBCs $152.3 \times 10^3 \mu\text{L}^{-1}$, RBCs $0.73 \times 10^3 \mu\text{L}^{-1}$, HGB 2.5gdL^{-1} , HCT 12%, MCV 164.4fL, MCH 34.2pg, MCHC 20.8gdL^{-1} , PLT $27 \times 10^3 \mu\text{L}^{-1}$, PCT 0.03% and PDW 8.7fL was measures in chronic Pb+Cd metal mixture stressed *O. niloticus*. Data obtained from experimental trial was analyzed statistically by using ANOVA and "LSD" test. In conclusions, result indicates that hematological parameters, selected in this study, revealed significant difference between control and metal mixture stressed fish group. The present study knowledge will be helpful for fishery biologists in the assessment of fish health and in monitoring stress responses.

COMPARATIVE GROWTH PERFORMANCE OF WILD AND HATCHERY PRODUCED SEED OF *LABEO ROHITA* IN SEMI-INTENSIVE CONDITIONS

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Fish meat is an essential constituent of human diet due to its rich source of animal proteins. The purpose of the present research work was to measure the comparative growth performance of

wild and hatchery produced seed of *Labeo rohita* in semi-intensive conditions. To achieve the goal of this study, 50 samples of proposed fish was collected from each selected private (Stiana fish seed hatchery and Shahkot fish seed hatchery) and public hatcheries (Faisalabad fish seed hatchery, Jhang fish seed hatchery). Proposed fish fingerlings were also collected from wild source (Chenab River). The experimental trial was conducted for 4 months in five earthen ponds one for each sampling site at Fisheries Research Farms, University of Agriculture, Faisalabad. All the limnological parameters were determined on weekly basis. To check the growth performance, various growth parameters such as weight gain, fork length, total length, feed conversion ratio, specific growth rate and condition factor was measured on fortnightly basis. Data obtained in this study were analyzed statistically by Mean \pm SD and ANOVA. Result of this study showed highest growth rate in *L. rohita* fingerlings collected from Stiana fish seed hatchery as compared to other sampling sites. Statistical analysis showed significant differences ($P < 0.05$) among different sources produced seed of *L. rohita* on the basis of various growth parameters.

FISH ECOLOGY AND BIODIVERSITY OF KHIRTHAR HILL TORRENT NAI GAJ DADU SINDH.

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Nai Gaj is one of the largest mountain torrents in south Pakistan. It is situated about 65 km (40 miles) north-west of Dadu city in Dadu District, Sindh Province of Pakistan. Nai Gaj Nala carries the second highest flow of water after the Indus River and it is the main source of fresh and unpolluted water for Manchar Lake. The main stream of Nai Gaj rises from North of Khuzdar and flows generally, southerly direction before turning sharply east, through a deep, narrow gorge in Kirthar Range into the Indus valley. There is a perennial flow of Nai Gaj which covers the area about 27 Km. This mountain torrent keeps on making the large number of depressions (Locally called as KHUMBH). This area is famous for the sport fishing and scene sighting. Due to the importance of this huge water body, it is aimed to clear the fish Biodiversity and ecology in Nai Gaj. The sampling was carried out from February to August 2015, however due to flood and other reasons fish samples were not conducted during the month of March and July. Fishing in this area was difficult due to tough geological position and depth of the water. Higher water temperature was lower in the month of February and higher in the month of August. The sampling was carried out with the various gears (Gill net, Seine net, Mosquito net). During the sampling total 19 species identified, preserved and studied. Among total 19 fish species seven families were observed during the research. Maximum species (10) were observed belonging to family Cyprinidae (*Labeo rohita*, *L. dyochelius*, *L. diplostomus*, *Cirrhinus reba*, *Tor macrolepis*, *Tor. Sp.2*, *Salmophasia bascilica*, *Puntius ticto*, *P. Sophore*); Three species were observed belonging to family Cobitidae (*Botia almorahae*, *Botia lohachata*, *Botia Kubotai*); family Sisoridae included only *Bagarius bagarius*; family Bagiridae included two species (*Rita rita*, *Mystus bleekeri*), two species in Siluridae (*Wallago attu*, *Ompok pabda*), one species in family Mastacembalidae (*Mastacembalus armatus*); and one species in family Cichilidae (*Oreochromis mossambicus*) were also identified. The study presents the first regular report of Mahasher (*Tor macrolepis*) from Sindh, and also presents the first report of *Botia kubotai* in Pakistan.

**THE STUDY OF CHEMICAL COMPOSITION AND MINERAL ANALYSIS OF SIX
COMMERCIALY IMPORTANT FISH SPECIES FROM THE RIVER
INDUS IN MIANWALI DISTRICT**

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Fish is a good source of animal protein and widely accepted due to its high taste, little cholesterol and tender meat. However, less number of consumers eats fish because of its nutritional value. It is therefore necessary to make information available to consumers and fishery workers on the nutritional contribution of some fish species in their daily diets. The present study was conducted to assess the chemical composition and minerals analysis of six commercially important fish species viz., *Labeo rohita*, *Channa marulius*, *Wallago attu*, *Clupisoma garua*, *Aorichthys aor sarwari*, and *Cyprinus carpio* from the River Indus in Mianwali District. These fish species were selected on the difference in their food niche including carnivores (*C. marulius*, *W. attu*, *A. sarwari*), herbivorous (*L. rohita*) and omnivorous (*C. carpio* and *C. garua*) species as well as on the preferences of consumers with respect to taste and economic cost of fish. The study followed a 6 x 3 factorial arrangement by involving six fish species with three dead weights (W1≤1.0, W2≤1.5 and W3≤2.0 Kg). The chemical composition of selected fish species was assessed by following AOAC (1990). Mineral profile was assessed by inductively coupled plasma - atomic emission spectroscopy (ICP-AES). The chemical composition of selected freshwater fish species showed high crude protein contents. Total fat contents were highest in *C. gaura* (27.43%) and lowest in *W. attu* (13.33%). The total fats were found proportional to body weight and this influences positive effect on the caloric value of the fish meat. The percentage ash contents were found to be 2.84 to 4.95%. The total carbohydrates showed a slight percentage of the total composition of the muscle and dry matter was found to be in the range of 18.89 to 22.13%. The order of mineral concentration in six fish species from the River Indus was P>K>Na>Mg>S>Ca>Fe>Zn>Cr>Pb>Ni>Mn>Hg. The bioaccumulation pattern of minerals in muscle tissue of different fish species was observed as: *C. carpio*>*L. rohita*>*A. aor sarwari*>*W.attu*>*C. marulius*>*C. gaura*. So, *C. carpio* being an omnivorous fish had the highest mineral load as compared to other freshwater fish species from the river Indus in Mianwali District. The selected major, micro and toxic minerals in six freshwater fish species were within the safe limits according to international standards. So fish in the study area is safe for human consumption. As all selected fish were good source of protein, fats, energy and minerals, so consumers who cannot afford costly fish can consume fish which will be available at cheaper price in the local market.

**INFLUENCE OF VITAMIN E WITH THREE LIPID SOURCES ON GROWTH AND
GILLS PEROXIDATION OF *LABEO ROHITA* FINGERLINGS**

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The aim of present research was to determine the effect of vitamin E with three lipid sources on growth and gills peroxidation in *Labeo rohita* fingerlings. Three dietary oils (fish oil,

canola oil and corn oil) were used to formulate three experimental diets which were further supplemented with two levels of vitamin E (125 and 1250 mg/kg), resulting in the formulation of six experimental diets. These diets were fed to fingerlings once a day to apparent satiation for 2 months. Water quality parameters i.e. temperature, pH and dissolved oxygen were monitored throughout the experiment. Weight gain was measured weekly during the whole experimental period to determine growth performance. At the end of the trial, fingerlings from each replicate were harvested to analyze hepatosomatic index, gills fatty acid profile, gills antioxidant enzymes activities and gills thiobarbituric acid reactive substances (TBARS). Supplementation of different oil sources and vitamin E did not show any effect ($p>0.05$) on growth performance of *Labeo rohita* fingerlings. Both supplements (oil from different sources and vitamin E) caused variations in the activities of antioxidant enzymes. Among oil sources, minimum antioxidant enzymes activities were recorded in fish fed corn oil supplemented diet compared to other dietary oils. Moreover, significant decrease in superoxide dismutase (SOD) and catalase (CAT) activities was recorded at mega dose of vitamin E (1250 mg/kg) compare to normal level (125 mg/kg). Minimum TBARS value was recorded in fish fed corn oil supplemented diet. Fatty acid profile was also significantly ($p<0.05$) affected by different oil sources, however, most of the fatty acid remain unaffected by vitamin E supplementation. Non-significant interactions between different oil sources and vitamin E levels were observed for growth performance, antioxidant enzyme activities and fatty acid profile of gills of *L. rohita* fingerlings. Conclusively, dietary oil source did not affect the growth performance of fingerlings and vitamin E behaved differently with different oils.

EFFECT OF STOCKING DENSITY ON GROWTH PERFORMANCE OF INDUS MAHSEER (*TOR MACROLEPIS*)

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Effects of stocking densities on the growth and survival of mahseer (*Tor macrolepis*) fingerlings were examined in glass aquaria. The experiment was conducted for two months in six aquarium having a size of 1x1.5x2 feet (height x width and length) each. Fingerlings were stocked at 10, 20 and 30 with replication, designated as treatment-1 (T1), treatment-2 (T2) and treatment-3 (T3) respectively. At stocking, all fingerlings were of same age group with a mean total body length of 7.8±2.15, 9.96±2.31, 5.68±1.25cm and weight of 5.38±3.57, 9.39±4.76, 2.96±0.72gm respectively. Fingerlings were fed with commercially available fish feed having 40% crude protein. Highest weight gain and total body length was observed in T1 and followed by T2 and T3. Survival rate was also observed higher in T1. Overall, the result shows the best growth performance and survival at a density level of 10 fingerlings. Further research should be done on stoking densities of this critically endangered species in ponds, cages and hapas to understand its biology and growth performance to enhance its ultimate production.

FISH DIVERSITY OF TANDA DAM KOHAT, KHYBER PAKHTUNKHWA

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The fish fauna of Tanda Dam Kohat was studied for 4 months i.e. from Jan to April 2015. During this study, collection was made from different sites of the dam. A total of 308 fish specimens were collected which comprised of 21 species. The reported fish species belong to 6 Orders, 7 families and 17 genera. Order Cypriniformes was represented by 2 families viz family Cyprinidae and Nemacheilidae. Family Cyprinidae was the most abundant family with 15 species viz *Labeo rohita*, *Labeo dyocheillus pakistanicus*, *Catla catla*, *Cirrhinus mrigala*, *Ctenopharyngodon idella*, *Hyphophthalmichthys molitrix*, *Cyprinus carpio*, *Puntius sophore*, *Puntius ticto*, *Puntius conchoni*, *Barilus pakistanicus*, *Barillius vagra*, *Tor macrolepis*, *Crossocheilus diplocheilus* and *Aspidoparia morar* while Family Nemacheilidae was represented by only one species i.e. *Schistura prashari*. The rest of the fish species namely *Chanda nama*, *Channa punctatus*, *Xenentodon cancila*, *Ompok pabda* and *Mastacembelus armatus* belonging to families viz. Chandidae, chanidae, Belonidae, Siluridae and mastacembelidae respectively was also reported. During the study period it was also detected that some species were present in large number while some species were present in less number. Fish species like *Hyphophthalmichthys molitrix*, *Cyprinus carpio*, *Labeo rohita*, *Aspidoparia morar*, *Xenentodon cancila*, *Mastacembelus armatus*, and *Crossocheilus diplocheilus* were very common and species like *Barilus pakistanicus*, *Barillius vagra*, *Tor macrolepis*, *Schistura Prashari*, *Chanda nama*, *Puntius ticto*, and *Puntius conchoni* were least common. During the study it was also found that the economically important stocking species such as *Hyphophthalmichthys molitrix*, *Cyprinus carpio*, and *Labeo rohita* are growing very well in the Tanda Dam while *Catla catla*, *Cirrhinus mrigala*, and *Ctenopharyngodon idella* are not successful in the dam and show very poor growth.

EFFECT OF REFRIGERATED STORAGE ON FILLET QUALITY OF ROHU (*LABEO ROHITA*) FED ON VITAMIN E SUPPLEMENTED DIETMUHAMMAD AFZAL,* AMBER IQBAL, SYED ZAKIR HUSSAIN SHAH
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The present research work was conducted to study the effect of vitamin E supplementation on fillet quality of rohu (*Labeo rohita*) during refrigerated storage. The duration of the experiment was 2 months. Graded levels of vitamin E were used to formulate six experimental diets namely D1, D2, D3, D4, D5 and D6. During feeding trial fish were fed once a day at 2% of its live wet weight. At the end of the experiment fish were sacrificed and their fillets were stored in refrigerator at -20°C temperature. The analysis of fatty acids profile, thiobarbituric acid reactive substances and assays of antioxidant enzyme activities were executed on fish fillet after 0, 15 and 30 days of frozen storage. The recorded data of the parameters were subjected to two way analysis of variance (ANOVA) to evaluate the treatments. The interaction of vitamin E and storage time was found to be significant for TBARS, antioxidant enzymes activities and fatty acids profile. In conclusion, the dietary vitamin E ameliorated the quality of fish fillets, as the lipid peroxidation was diminished, the enzyme activities were increased and improved ratios of polyunsaturated fatty acids were acquired.

EVALUATION OF WATER HYACINTH (*EICHHORNIA CRASSIPES*) SUPPLEMENTED DIETS ON THE GROWTH, DIGESTIBILITY AND HISTOLOGY OF GRASS CARP (*CTENOPHARYNGODON IDELLA*) FINGERLINGS

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Study was conducted to evaluate the potential of water hyacinth as aqua feed for grass carp (*Ctenopharyngodon idella*) and its effects on growth, body composition and histology. Fish were stocked in eight glass aquaria having dimensions of 3' x 1.5' x 2' @ 20fish/ aquaria. Three iso-proteinous diets (30%) were prepared with the addition of different parts of sun dried water hyacinth i.e., whole plant meal (WPM), leaf meal (LM) and root meal (RM) and control diet. All experimental diets were replicated twice per treatment. The design of the experiment was CRD and the results were analyzed using ANOVA technique. Results of the study revealed a significant ($P \leq 0.05$) difference in weight gain and length among treatments. Higher weight gain was observed in LM and lower on RM diets. Crude protein contents of fish fed WPM was significantly higher ($P \leq 0.05$) followed by LM than RM and control diets. Crude fat was significantly higher in fish fed by RM followed by LM while ash contents were significantly higher in control than treated. Nutrient digestibility in case of CP was significantly higher ($P \leq 0.05$) for WPM, LM and control than RM while fat digestibility was significantly higher ($P \leq 0.05$) in RM diet followed by LM than WPM and control. Histological studies showed no abnormalities in liver and kidney due to incorporation of water hyacinth. In conclusion, LM based diets were found most suitable for the growth without any adverse effects on the histo-pathological disorders in experimental fish.

EFFECTS OF SYNTHETIC HORMONES ON THE CAPTIVE BREEDING OF SILVER CARP AND GRASS CARP

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The present study was conducted to know the comparative effect of two synthetic hormones i.e. pituitary gland (PG) extract and Ovaprim on the captive breeding of Silver Carp (*Hypophthalmichthys molitrix*) and Grass carp (*Ctenopharyngodon idella*) The PGE hormone was given at the rate of 7 mg/kg of body Weight for females and 3.5 mg/kg of body weight for males. In contrast, Ovaprim was administered at the rate of 0.5 ml/kg of body weight and 0.25 ml/kg of body weight for females and males, respectively in present study Ovaprim show higher success rates for induced spawning as compare to (PG).The percentage of ovulation ranged (90 - 95%) was found with ovaprim treatment. and (70 - 80%) with pituitary extract treatment The percentage of fertilization ranged (79.6 - 83.1%) was found with ovaprim treatment. and (60.3 - 71.7%) with pituitary extract treatment. The percentage hatchling ranged (70.2 - 75.1%) with ovaprim treatment and (54.3 - 60.4%) with pituitary extract treatment. In summary, Ovaprim shows better results in induced spawning as compare to (PG).

A GENERALIZED STRUCTURE OF CTENOID SCALE OF *SCATOPHAGUS ARGUS* (LINNAEUS, 1766) FAMILY SCATOPHAGIDAE, USING LIGHT MICROSCOPY

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This study provides basic information about the structure of scales of spotted scat, *Scatophagus argus* (Linnaeus, 1766). Specimens of *S. argus* were collected from the commercial landing sites at Karachi fish harbour and Korangi fish harbour, Pakistan. Three regions of fish body (*i.e.*, head, body and caudal region) were selected to study the variations in scale structure of *S. argus*. The result of light-microscopy shows that *S. argus* has spinoid type of ctenoid scales. Focus was absent in the scale and no or very few numbers of radii were observed only at the anterior margin of the scale and rarely in lateral fields of the scale. The result of this study confirms the authenticity of the scale parameters in systematic classification of fishes.

NEW SPECIES *WITENBERGIA MYSTUSI* OF GENUS *WITENBERGIA* VAZ, 1932 FROM RIVER INDUS CATFISH *MYSTUS CAVASIUS* SINDH, PAKISTAN.

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Present work on helminth parasites of host catfish *Mystus cavasius* from river Indus Jamshoro district, Sindh, Pakistan. Total 67 host fishes were collected from different habitats of study area. During examination 26 trematodes were collected from intestine of *Mystus cavasius*. These trematodes belong to genus *witenbergi* Vaz, 1932 and differs from other species of genus on the basis of different body shape and size, oral sucker cup shape, ventral sucker rounded, prepharynx elongate, esophagus much longer, testes rounded to oval in shape and half overlapped by uterus, seminal vesicle posterior to ventral sucker and curved elongate in shape, ovary rounded and postequatorial, seminal receptacle oval in shape and preovarian, uterus in the form of loops and much compact, identified as new species *Witenbergia mystusi*. The name of new species *Witenbergia mystusi* refers on the basis of host fish *Mystus cavasius* from which species collected.

DETECTION OF MYCOFLORA FROM FISHES OF KARACHI FISH HARBOUR

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In our study we collected fourteen different genera of fishes *Acanthopagrus* sp., *Parastromateus niger*, *Nemipterus* sp., *Pampus argenteus*, *Ilisha* sp., *Alepes djedaba*, *Epinephelus* sp., *Teraponjarbua*, *Terapon puta*, *Scomberomorus koreanus*, *Epinephelus coioides*, *Lutjanus* sp., *Pomadasys* sp. and *Lutjanus johnii* from Karachi fish harbour and isolated five different species of fungi *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium* sp. and *Fusarium* sp. *Aspergillus flavus* was a most dominant fungi as compared to other species of

fungi. Current research reported that only four genera of fungi isolated from fishes of Kemari harbour which represent that fresh fishes were not contaminated by incidence of fungi. The present study denotes that fresh fish consumption is safe and provide us high quality protein and amino acids as compared to stored fishes.

RESENT STATUS OF PALLA, *TENUALOSA ILISHA*

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The River shed locally known as Palla, *Tenualosa ilisha* is anadromous migratory clupeid fish found in Pakistan, India, Bangladesh, Iran, Myanmar, Kuwait and Malaysia. In Pakistan River Indus supports it run, starts from May to August for breeding and covers about (180 river miles) up to Ghulam Muhammad Barrage, near Jamshoro. The present status has been determined in relation to the downstream flow of River Indus. The catch of this commercially important fish has drastically declined with the discharge. Earlier its catch was reported more than 1800 metric tons during 1980's and declined up to 200 metric ton by 2002. It has been also reported that there are two types of Palla (*T.ilisha*) are available in River Indus, one breeds in summer (during monsoon) and other breeds in winter (during December to March). It is concluded that if the downstream flow of River Indus continued throughout the year this fish could breed twice in a year which enable us to save this from extinction.

EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL OF *TILAPIA CHITRALADA* REARED IN CEMENTED CISTERNS

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Present study was taken in to account on the effect of stocking density on growth and survival of *Tilapia chitralada* reared in cemented cisterns department of Freshwater Biology and Fisheries, University of Sindh, Jamshoro. Thirteen cemented cisterns (size 1.25 m² each) were maintained and designs 10, 15, 20 and 25 fish per cistern as Treatment I, II, III and IV replicated thrice. It was noticed from the results of present study on stocking density of *T. chatralada* that the highest weight gain, specific growth rates and survival rate was observed in low stocking density treatment I (40.68g, 1.28 and 100%) respectively followed by treatment II (30.46g, 1.16 and 90%) and lowest in Treatment III (22.91g, 1.12 and 80%). Feed conversation ratio was recorded low in Treatment I (5.0) and production was found higher in high density Treatment IV (0.30) and lowest in TI (0.18). All the physicochemical parameters were found to be in suitable ranges. It was concluded that the low density is best for culture of *Tilapia nilotica* in cemented cisterns.

**BIO-CHEMICAL COMPOSITION OF CATFISH, *RITA RITA* FROM
INDUS RIVER NEAR JAMSHORO**P. KHAN,¹ N.T. NAREJO,¹ S. JALBANI,¹ A.J. LAGHARI² AND F. MEMON³¹Department of Freshwater Biology and Fisheries, University of Sindh, Jamshoro²Center for Advanced Research in Chemical Sciences, University of Sindh, Jamshoro³Department of Physiology, University of Sindh, Jamshoro

For biochemical composition of catfish *Rita rita*, 60 specimens ranged from 10.1- 40.0 cm and 75.0- 632.0g in total length and weight respectively were collected from Indus River near Jamshoro during November 2015 - January 2016. The study on biochemical composition was enumerated from head, trunk and tail region at 3 different length groups (small, medium and large). Results of study revealed that highest % of moisture was observed from head region (84.5) followed by tail region (83.6) and lowest was observed (82.5) in trunk region, in case of % protein highest was observed from tail region (12.1) followed by trunk region (10.43) and lowest in head region (8.33), % fat contents was observed highest in head region (6.83) followed by trunk region (6.7) lowest in tail region (3.7) while ash was observed highest in trunk region (3.1) followed by tail region (2.7) and lowest in head region (2.0) at each length group.

**EFFECT OF DIFFERENT SALINITY LEVEL ON BREEDING, FERTILIZATION,
HATCHING AND SURVIVAL OF NILE TILAPIA, *OREOCHROMIS NILOTICUS*
(LINNAEUS, 1758) IN CAPTIVITY**ABDUL MALIK,^{1,2*} GHULAM ABBAS,¹ SARA FERRANDO³AND LORENZO GALLUS³¹Centre of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan²Directorate of Fisheries Sindh, Livestock & Fisheries Department, Govt. of Sindh, Pakistan³Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, Italy

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This study was conducted to assess optimal salinity level among 0‰, 5‰, 10‰, 15‰, 20‰ and 25‰ for successful breeding of Nile tilapia, *Oreochromis niloticus*. The duration of study was 56 days. Brooders (48) having mean weight (male 162±0.2g and female 160±2.5g) were selected and stocked into hapa nets in 12 fiberglass tanks (2000-liter). Ratio among male and female was 1:3. They were fed with commercial floating pelleted feed constituting 35% crude protein with 2% body weight twice a day. Eggs were collected on weekly basis by cultch removal method. Results showed that the highest fecundity, fertility, hatchability and survival of fry were obtained on salinity of 0‰ to 15‰ and significantly decreased on 20‰ and 25‰. The eggs per gram body weight were also recorded in all treatments and highest eggs were obtained *i.e.* 4.0 to 4.3 per female on 0‰ to 15‰. Water temperature (28.2±0.2°C), dissolved oxygen (6.3±0.1 mg/L), pH (6.6±0.1) and ammonia (less than 0.02±0.005 mg/L) were monitored throughout the study period. Water quality parameters remained within the recommended range. Our results suggest that Nile tilapia, *O. niloticus* may give maximum eggs up to 15‰ salinity with 92% survival of fry.

IMPACT OF WATER QUALITY PARAMETERS ON THE GROWTH OF ORNAMENTAL FISHES

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In the present investigation, impact of some water quality parameters temperature, salinity, pH, dissolve oxygen, ammonia were examined to observed their impact on the growth of ornamental fishes in aquarium during the period extends from June 2016 to December 2016. In this study, the changes in temperature, salinity and pH of water were calculated seasonally i.e. June to September (warmer month) and October to December (cooler month). The obtained results revealed that the changes were seen in growth as well as in behavior of these fishes when they were fed two times per day. For maximum growth of fishes, we need to sustain each selected water quality parameters in their optimum ranges or ranges as required by the cultural species.

COMPARATIVE GENOTOXIC STUDY OF MAJOR INDIAN CARP *LABEO ROHITA* AT RAVI, HEAD BULLOKI (PATTOKI) AND SATLUJ, HEAD SULEIMANKI

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In relevance with environmental toxicity, expression of toxicity symptoms in the form of genetic damage and nuclear abnormalities like micronuclei under stress of heavy metals in fish is quite supportive. Comet assay and Micronucleus test are advantageous biomonitoring tools for detecting damage on level of individual cell. Present research work was conducted to estimate comparative genotoxicity in *Labeo rohita* by emphasizing on DNA damage (Tail Length) and nuclear abnormalities by Comet assay and Micronucleus Assay in research laboratory of department of Zoology, GCU, Faisalabad. Fresh fish samples weighing 1kg, 1.5kg and 2kg were captured from four sites i.e. **BU**, **BD**, **SU**, **SD**, blood was drawn into EDTA viles and kept in chilled ice box. Later blood samples were refrigerated at 4°C in laboratory. Comet Assay and Micronucleus assay were applied with slight modifications to all blood samples, slides were examined, comet tail length was scored and cells with micronuclei were counted. Heavy metals i.e. Cu, Cr, Zn, Ni, Pb, Mn and Fe in water and sediment of sites were analyzed by atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). Mean weight of *Labeo rohita*, **BU**: 1391.90±279.8, **BD**: 1442.93±363.71, **SU**: 1593.66±337.24 and **SD**: 1653.26±436.56. Mean length measured **BU**: 40.3±2.63, **BD**: 42.0±2.72, **SU**: 44.7±4.60, **SD**: 44±4.7. Mean comet tail length **BU**: 8.7±1.11, **BD**: 7.56±0.86, **SU**: 13.67±3.32, **SD**: 12.31±2.82. Micronuclei counted **BU**: 3.12±1.24, **BD**: 4±1.60, **SU**: 3.75±1.98, **SD**: 3.87±1.45. Heavy metal concentration in water samples **BU**: Fe> Zn> Mn> Pb> Cr> Cu> Ni, **BD**: Fe> Pb> Mn> Zn> Ni> Cr> Cu, **SU**: Fe>Pb>Mn>Zn>Cr>Ni>Cu, **SD**: Fe> Mn> Zn> Ni> Cr> Cu>, in sediment samples recorded **BU**:Fe>Mn>Zn>Pb>Cr>Cu>Ni, **BD**: Fe> Mn> Zn> Cr> Pb> Cu> Ni, **SU**: Mn> Zn> Fe> Cr> Cu> Pb> Ni, **SD**: Mn> Zn> Pb> Cu> Fe> Cr> Ni. Conclusively, Relative genotoxicity between Satluj and Ravi indicates high genotoxic potential in *Labeo rohita* of Suleimanki region as it receives polluted water from the ravi on one side and from India on the other side. Natural and anthropogenic sources introduce heavy metals in soil and in fresh water thus disturbing the water quality and polluting the natural aquatic ecosystem creating danger for the fish.

**ISH DIVERSITY AND DISTRIBUTION OF RIVER PANJKORA AT DIR DISTRICTS
(UPPER AND LOWER), KHYBER PAKHTUNKHWA, PAKISTAN**

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The present study was conducted to determine the fish fauna of River Panjkora at Dir districts (upper and lower), situated in Northeast of Khyber Pakhtunkhwa, Pakistan. The survey was conducted from March to September 2016 at seven sampling sites to ascertain fish diversity and their distribution pattern. A total 724 specimens were collected using a variety of fish nets and identifications of these fishes were made by using standard taxonomic keys on the basis of morphometric characters. Eighteen species belonging to 14 genera and 5 families were recorded. Eight species were commercially important. Nine species were indigenous, eight were endemic and one species *Carassius auratus* was exotic were reported in the River Panjkora. The fish fauna of river Panjkora was dominated by species belonging to the family Cyprinidae (56.49%) followed by Nemacheilidae (24.44%). Families Channidae, Sisoridae and Mastacembelidae constituted 10.63%, 7.04% and 1.38% respectively. Most dominant species was *Schizothorax plagiostomus* (16.57%) followed by *Carassius auratus* (11.87%) and *Racoma labiata* (9.66%). These species were abundant especially during the months of April, May and June. The least abundant species were *Glyptothorax punjabensis*, *Glyptothorax sufii* and *Mastacembelus armatus* and constitute 2.48%, 2.20% and 1.38% of the total. Over all Simpson's diversity value (1-D= 0.919), Simpson's Reciprocal index value (1/D= 12.3876) and (Shannon's index = 2.68) indicates that river Panjkora is quite diverse concerning fish species richness and evenness. *Schizothorax plagiostomus* was the top one distributed in all the sites studied followed by *Schizothorax esocinus* found in six sites, *Carassius auratus* found in four sites, *Garra gotyla*, *Nangra robusta*, *Acanthocobitis botia*, *Schistura prashari* and *Triplophysa microps* are equally distributed in three sites. *Tor putitora*, *Racoma labiata*, *Channa gachua*, *Channa punctata* were found in two sites while *Glyptothorax sufii*, *Glyptothorax punjabensis*, *Barilius vagra*, *Puntius ticto*, *Schistura alepidota* and *Mastacembelus armatus* were found only in one site of the study area.

**CROSS BREEDING TECHNIQUES BETWEEN THE ORNAMENTAL FISHES AND
OBSERVING THEIR VARYING MORPHOLOGY**

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The main object of our present study was to maintain, rear and to achieve the new generation from the cross breeding. We have put Red platy, Selphon Mulley, Balloon Mulley and sword tails in a glass tank containing 200 liter water and fed with diet of dried worms and tubifex worms with proper aeration regularly. Water quality parameters were within the tolerable limits for fish throughout the experiment as the water temperature was maintained at 29.3±0.76°C (mean±SD), pH was 7.2±0.51 and dissolved oxygen ranged from 4.0 to 7.5 ml/l with a mean of 6.2±0.95 ml/l during the study period. We obtain the younger ones from cross breed of Selphon

Mulley and balloon Mulley which are distinguish by their physical appearance like color and design pattern and by the caudal fin arrangement. In total we have obtain the 57 young ones which have variations in their color and designs and arrangement of fins.

SEASONAL VARIATIONS IN NUTRITIVE VALUE AND BIOCHEMICAL COMPOSITION OF COMMERCIALY AVAILABLE FEED INGREDIENTS AND SELECTED AQUATIC PLANTS FROM KASUR, PUNJAB, PAKISTAN

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The present study extended from January through March 2016 was conducted to assess the nutritive value and biochemical composition of selected feed ingredients and aquatic plants through proximate analysis. All feed ingredients and aquatic plants were dried and then subjected to analysis. Moisture content was higher in rice bran (10.47±0.25) while lower in guar meal (3.53±0.07). Similarly, vellesneria (5.22±0.08) was high in moister contents as compare to other aquatic plants. Maximum crude protein was recorded in fish meal (64.83±0.77) and vallesneria (28.23±0.25) whereas maximum crude fat was obtained in cotton seed meal (10.27±0.15) and in typha (7.22±0.08). Fiber and ash content were high in rice bran (22.53±0.45) and (15.42±0.13) respectively. Typha was rich in fiber (14.71±0.10) and phragmite has higher ash contents (10.75±0.25). NFE was higher in corn meal (79.77±0.29) and pickerel weed (74.15±0.05). These feed ingredients and aquatic plants have high content of nutrients especially protein which is the major requirement of fish and other animals. Exploitation of feed ingredients and aquatic plants are economical to make additional feed for aquaculture production.

COMPARATIVE ANALYSIS OF WATER QUALITY OF SELECTED PONDS FOR THE SURVIVAL OF TILAPIA

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The study was conducted for the qualitative and quantitative comparison of fresh water pond and treated waste water ponds to know the survival rate of Tilapia. The water system plays a pivotal role in the development of the adaptive behavior, an important circulation of nutrients, buoyancy and electrolytes etc., in the survival rate of tilapia. Rate of physiological functions in fish is directly affected by changes in temperature due to poikilothermic nature of fish. Photoperiod

impact the immune response of fish by increasing the amount of circulating IgM and activity of lysozyme. Tilapia is resistant in harsh conditions, fast reproduction ability, easily cultured in treated waste water. The project outcome was based to determine the comparative water quality of the fresh water pond and the treated waste water ponds. The qualitative and quantitative analysis indicated that in the treated waste water pond the amount of ammonia (5.24 ± 0.65), pH (9.65 ± 0.15), electricity conductivity (3.31 ± 0.64), total hardness (269.77 ± 12.59), total alkalinity (373.90 ± 11.93) and different organic compounds were significantly high in concentration that were not supportive for the survival of the tilapia.

AN ASSESSMENT OF BYCATCH OF HIGH SEAS GILLNET FISHERIES OF PAKISTAN

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Gillnet operations in the offshore waters of Pakistan including Exclusive Economic Zone (EEZ) and the Area Beyond National Jurisdiction (ABNJ) are aimed to target 8 species of tuna. This fishery contribute substantially to landings of a number of fish species in Pakistan. In addition to tuna, a number of commercially important finfish species (excluding sharks) are also caught. Among these bycatch species billfishes, Spanish mackerels, queenfishes and dolphinfishes are dominating. Billfishes represented by Indo-Pacific sailfish (*Istiophorus platypterus*) and black Marlin (*Makaira indica*) are dominating in the bycatch almost throughout the year especially during winter months. Common dolphinfish (*Coryphaena hippurus*) is another species which is caught throughout the year and contributing substantially to total landings of pelagic fisheries of Pakistan. Unprecedented increase in the bycatches of unicorn leatherjacket filefish (*Alutrea monoceros*), rough triggerfish (*Canthidermis maculata*) and largescale triggerfish (*Canthidermis macrolepis*) is of great interest, as these species were not reported in landings of tuna gillnetters during last decades.

SPATIAL AND TEMPORAL DISTRIBUTION OF ROUGH TRIGGERFISH (*CANTHIDERMIS MACULATA*) AND LARGESCALE TRIGGERFISH (*C. MACROLEPIS*) IN THE ARABIAN SEA

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Two species of family Balistidae i.e. *Canthidermis maculata* (Bloch 1786) and *C. macrolepis* (Boulenger, 1888) are known to occur in the offshore waters of Pakistan. Studied carried out since 2012 reveals that this species is found in mainly in the offshore waters at Khori Great Banks on Sindh coast and off Sapat/Malan along Balochistan coast. Although it is found throughout the year but more abundant during winter season (November through February). This species is found to be associated with drifting objects. The paper describes their size distribution and details of morphological features of the species.

POPULATION GENETIC STRUCTURE OF ROHU, *LABEO ROHITA* HATCHERY STOCKS USING FIFTEEN MICROSATELLITE LOCI

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Inappropriate hatchery stock propagation practices are compromising the genetic integrity of distinct local populations. The present study examined the genetic structure of fish *Labeo rohita* in ten selected hatchery populations throughout Punjab as influenced by anthropogenic interventions and artificial propagation programmes. The fish specimens were collected from the target sites, 25 from each hatchery population originating from different districts of Punjab. Total fifteen microsatellite loci with varying levels of polymorphism were employed to examine genetic differentiation and population structure of the species within sampling localities. The number of alleles per locus varied between 2 and 7 with an average of 4.0 alleles per locus. Overall, low to moderate level of heterozygosity was observed in all the hatchery populations. Observed heterozygosity varied from 0.4488 up to a maximum of 0.5667. The average value of F_{IS} ranged from 0.144 to 0.337, reflecting significant level of inbreeding in most of the hatchery populations. Significant deviations from Hardy-Weinberg equilibrium ($P < 0.05$, $P < 0.01$, $P < 0.001$) were observed in about 72% of the total locus-population combination tests with apparent heterozygote deficits. This heterozygote deficit might be attributed to poor broodstock management practices in the hatcheries. The AMOVA revealed two major components of genetic variation; within-population (93.47%) and among-populations (6.04%). Based on pairwise F_{ST} and unbiased genetic distance, low-to-moderate level of differentiation was found among the samples from scattered hatchery populations. The UPGMA dendrogram clustered the populations onto two major branches, surprisingly departing from their geographical origin. Employing more polymorphic markers and exhaustive sampling over a wide geographical scale may explore a fine-scale genetic structure of *L. rohita* populations in future.

STATUS OF FISH DIVERSITY OF ISLAM BARRAGE, RIVER SUTLEJ, PUNJAB, PAKISTAN

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Islam barrage is used for irrigation and flood control. It is situated near Vehari and Hasilpur. It was completed in 1927. The old structure of barrage has been documented as poor since 1998. Due to lack of interest for reconstruction of barrage, and India releases a large amount of water in river Sutlej; have threats to local people of the area. The objective of the study was to know the diversity of fishes of Islam Barrage, river Sutlej, Punjab, Pakistan. Fish samples were collected by direct and indirect methods to know the fish diversity of this area. During the survey of Islam barrage 23 species were recorded from this area. Most abundant species of this area were mali, rohu, sunni, shinghara, tilapia, jhalli, dola, thaila and singhi. Statistical analysis showed that Shannon-Weiner diversity index (H') of this area was as 1.31745.

**STUDY OF FILLET QUALITY OF *LABEO ROHITA* FINGERLINGS FED *MYO* -
INOSITOL SUPPLEMENTED PRACTICAL DIET**

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Present research work was planned to study the fillet quality of rohu (*Labeo rohita*) fingerlings fed *myo*-inositol supplemented practical diet. This experiment was conducted for 60 days. Five experimental diets namely D1, D2, D3, D4 and D5 were formulated by supplementing *myo*-inositol at the level of 0, 100, 200, 400 and 800 mg/kg diet, respectively. Practical diets were allotted in duplicates with the stocking density of 15 fish per tank. During feeding trial, fish were fed once a day at 2% of its live wet weight. At the end of the experiment, 10 fingerlings from each replicate were sacrificed and their fillets were stored in refrigerator at -20°C temperature. At the end of the trial, muscles proximate composition, fatty acid profile, thiobarbituric acid reactive substances (TBARS), anti-oxidant enzymes and intestinal digestive enzymes activities were recorded. Resulting data were analyzed statistically by using one-way analysis of variance (ANOVA). The results indicated that *myo*-inositol supplementation did not show any significant effect on proximate composition. The thiobarbituric reactive substances (TBARS) were significantly ($p < 0.05$) reduced and the enzymes activities (SOD, CAT and GPX) were improved in fillets by increasing *myo*-inositol supplementation. The activities of intestinal digestive enzymes (protease and lipase) were also elevated significantly ($p < 0.05$) against the addition of *myo*-inositol supplementation. Fatty acid profile indicated that by increasing *myo*-inositol supplementation 14:0 n-0, 16:0 n-0, 22:5 n-3, 22:6, n-3 fatty acids and n-3/n-6, ARA/EPA and EPA/DHA ratios did not show any significant response against *myo*-inositol supplementation. In conclusion, *myo*-inositol supplemented diet improved the antioxidant status and intestinal digestive enzymes activities in *Labeo rohita* fingerlings.

**ASSESSMENT OF FISHERIES RESOURCES IN LAKES AND STREAMS OF GILGIT-
BALTISTAN (GB) AND PROSPECTS FOR ITS SUSTAINABLE CONSERVATION**

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Developmental activities have been tremendously increased in GB since last decade, which putting a pressure on freshwater fisheries resources. The problem is further intensified due to climate change and natural disasters. Both anthropogenic and natural factors are contributing in deteriorating of fisheries resources. There is limited and scanty information is available about the fisheries resources of Gilgit Baltistan. To bridge up the gaps of information related to fisheries resources, present study was designed by GB Fisheries Department and WWF Gilgit-Baltistan to highlight the existing status of fisheries resources in some selected lakes and streams. In this

regard, five lakes (Hundrab, Darle, Jarbazoo and Naltar) and two streams (Ghizar river and Khanbari Nala) in GB were sampled for the freshwater fish species and their sustainable conservation. A variety of fish sampling methods viz; angling, cast and gill netting were applied to study the fish fauna. All fish specimens were identified using www.fishbase.org and length weight parameters were also measured. A total of 11 species were sampled with abundant species *Salmo trutta fario* (Brown trout) an exotic fish and well established in lakes of GB network of streams. *Oncorhynchus mykiss* (Rainbow trout) an other exotic species present in the lakes and stream segments where it was stocked but fail to breed in natural environment, however, frequently stocked for commercial purposes. Among nine native fish species were collected from study area. Native cyprinids species viz; *Schizothorax plagiostomus*, *Schizothorax esocinus* and *Schizothorax skurduensis*, were the species commonly distributed in stream network of GB. The most productive lake was Hundrab and Ghashu lakes with good fish stocking, whereas most diverse lake was Jarbazoo lake and rest of two Darle and Naltar lakes were less diverse and commercially less productive. High fish stocking in Hundrab Lake was due to ban on all kinds of fishing imposed by GB Fisheries Department since last three years with the cooperation of local community. Jarbazoo lake represents the four species and has great potential to stock both native and exotic fishes Ghazir River and Khunburi Nala both act as breeding site of native fishes during monsoon season. Rapid construction of road networks, water diversions for irrigation and hydropower stations and dam construction are the major threat to habitat and breeding grounds. To maintain the harvestable populations of trout species in lakes for protein demands and conservation of native fishes in river and stream, there is need of continuous monitoring and management of fisheries resources in a sustainable way.

FISH POTENTIAL FROM KAREZ SARAWAN, PANJGOOR BALCHISTAN

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Present study was undertaken to investigate fish potential from Karez Sarawan, Balochistan. In total 2694 specimen of different fish species were collected ranged between 5.5 to 13.08 cm and 0.9-22.06 g, respectively. It was observed that the fish potential of Karez Sarawan consists of 3 families, 4 genera and 7 species namely *Cyprinion watsoni*, *Channa striata*, *Labeo buggut*, *Labeo bata*, *Schizothorax* sp. *Aphanius ginaonis*, *Aphanius dispar*. Among the species *Cyprinion watsoni* was considered as the most abundant and constitutes about (70.68%) followed by *Schizothorax* sp. and *Labeo buggut* (11%) of the total catch. The length-weight relationship values of the above fish were calculated and observed from the equation that *Labeo bata* found to be in ideal condition, *C. striata*, *C. watsoni* and *Labeo buggut* found in satisfactory growth respectively and poor growth was noticed in case of *Schizothorax* sp. from Karez Sarawan. The Simpson's biodiversity index (I-D = 0.481) shows that the Karez has low ichthyic diversity. It is an intense need to monitor water quality parameters regularly and stock fish in the Karez to improve and enhance the diversity. Finally it was concluded that the environment of Karez Sarawan favors the potential of economically important carp *L. bata*.

**PROBIOTICS AS A GROWTH PROMOTER AND
IMMUNOSTIMULANT IN *CATLA CATLA***

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This study was performed to determine the impact of probiotics on growth and immune response of *Catla catla*. The fish with average body weight was stocked in earthen pond at a density of 20 fish per pond and fed with two commercial probiotics (Protexin and Yeast) for a period of 120 days @ 4% of wet body weight once a day, in two equal installments. The fish were distributed randomly into four treatment groups, T₁ (Protexin 0.2%), T₂ (Protexin 0.5%), T₃ (Yeast 0.2%) and T₄ (Yeast 0.5%). The growth parameters of *C. catla* (body weight, fork length, total length, FCR, and SGR) were measured fortnightly. Blood samples were collected at the intervals of 0, 30, 60, 90 and 120 days to determine hemato-immunological parameters. Fish growth in terms of weight gain was significantly higher in T₂ followed by T₁, T₄ and T₃. The increase in fork and total length also followed the same trend as weight. Fish showed significantly better FCR and SGR for T₂ and T₁ as compared to other treatments. Fish in T₂ group showed maximum hemoglobin contents, hematocrit contents, red blood cells count, white blood cells count, mean cell hemoglobin, mean cell hemoglobin concentration, lymphocyte and monocyte than other treatments. The results proved that T₂ (0.5% Protexin) showed maximum growth promotion and immunostimulation in *C. catla*.

**STUDY OF SELECTED WATER QUALITY PARAMETERS OF RIVER INDUS AT
GHAZI, HUND AND KUND, DISTRICT SWABI, KHYBER PAKHTUNKHWA,
PAKISTAN**

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The present study was designed to scrutinize the selected water quality parameters of Indus River at three sites that are Site A, B and C named as Ghazi, Hund and Kund respectively of District Swabi, KHK-Pakistan. The purpose of the study was to evaluate the suitability of water for survival, growth and reproduction of fishes. The present study was conducted for three months that are January, March and June (2016). From each site, in each study month, three samples were collected and for analyzing samples were brought to PCSIR Peshawar. Studies Parameters were pH, Electrical conductivity, Total Dissolved Solids, Total Suspended Solids, Total Hardness, Calcium Hardness, Magnesium Hardness, Total Alkalinity, P-Alkalinity, Chloride, Potassium, Sodium, and Sulphate. Concentration of these parameters were, 7.95, 295ppm, 180ppm, 30.77ppm, 134.02ppm, 94.51ppm, 45.05ppm, 140.35ppm, 45.33ppm, 37.29ppm, 6.74ppm, 19.75ppm and 45.10ppm respectively. From the above observations it was concluded that the concentration of all the studied parameters at each site were in normal range and having no adverse effect on fish survival, growth and reproduction.

**ECOLOGICAL LINKAGES WITH WATER QUALITY ASSESSMENT
IN CHASHMA WETLANDS**

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This paper gives some findings of the positive impacts of the migratory birds, on the water quality, submerged flora and the aquatic macroinvertebrates of the main left marginal water reservoir of Chashma Barrage at river Indus, in district Mianwali, Pakistan. The study was conducted from first week of March to last week of July 2015, in four field trips. The water quality was measured on the spot with 'Hydrolab' and the water samples were taken from surface as well as bottom of the pond. The samples of the macroinvertebrates and the flora, both floating and bottom were collected from the same spots. This reservoir gets huge influxes of migratory waterfowl during the winter months. These may be as many as 60,000 coots and ducks in peak winter months and over 100,000 in February, when the flocks grow in size for migration back to their wintering areas. Additionally, hundreds of gulls and terns, feed on surfacing small fish, flying low over the water, while almost the same number of cormorants also dive under water to catch small fish. The constant addition of bird droppings throughout the winter months enhances the fertility of water with the gradual increase of water temperature in post winter season. This was assessed through sampling during the spring and summer months. A gradual rise in Nitrates, Nitrites and Phosphates was found in the water due to the rise in the water temperature which enhanced the fermentation of the huge amounts of the bird droppings. The aquatic floral and macroinvertebrates samples showed increase in amount and diversity. Similar studies have been conducted in other countries where birds' droppings increased the total nitrogen and phosphorus in pond water till the extent of eutrophication. Positive SPSS (Statistical Package for the Social Sciences) regression results of aquatic flora and fauna with water quality parameters in Chashma wetlands showed that water productivity due to nitrates, nitrites and phosphates flourished growth and development of aquatic flora and fauna in the wetland. Rise in water temperature due to fermentation of droppings added during roosting of birds arriving in millions from November to February and during their departure in the end of February, enhanced the water fertility. Henceforth floral biomass was found high in March, April and June. The dredged floral biomass was higher than floating flora. This reservoir is important source of revenue generation through commercial fishing. A strong indication of positive correlation between the aquatic flora and macroinvertebrate fauna was found which shows their interdependence in the food chain of the water reservoir.

**DISTRIBUTION AND DENSITY OF PELAGIC COPEPODS COLLECTED BY THE
NORTH ARABIAN SEA ECOLOGICAL AND ENVIRONMENTAL RESEARCH
(NASEER) CRUISE 1 IN THE NORTHERN ARABIAN SEA**

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Marine Reference Collection and Resource Centre, University Of Karachi

The present paper is based on the study of pelagic copepods from the Arabian Sea. The materials were collected under the North Arabian Sea Ecological and Environmental Research (NASEER) Cruise I (January 7 to 22, 1992) and during the US Office of Naval Research (ONR) project (1993-1995). In all 75.30% age copepods were taken in. In the NASEER I sample four

major groups were separated i.e. Calanoida, Harpacticoida, Cyclopoida and Poecilostomatoida. In all 27 genera and 39 species have been identified. The study deals with the distribution, density and abundance (%age) of these four major groups in the northern Arabian Sea. The abundance of different taxa is discussed with association to time of sampling and distance and distance of sampling station from the coasts. The species are also illustrated. The Calanoida is the most abundant order. It comprised of 75.86% of the total copepods, belonging to the eleven families viz. to Metridinidae, Acartiidae, Candaciidae, Centropagiae, Pontellidae, Temoridae, Euchaetidae, Eucalanidae, Calanidae and Paracalanidae with twenty-seven species. Poecilostomatoida were the second most abundant group, comprising of 20.08% of the total copepods and represented in NASEER I samples by three families viz. Corycaeidae, Oncaeidae and Sapphirinidae with eight species. The Cyclopoida comprise of 3.64% of the total copepod with a single family Oithonidae and a single species. The population of Harpacticoida was very thin, only 0.41% of in the samples with three families viz. Ectinosomatidae, Clytemnestridae and Miraciidae and four species.

WATER QUALITY ANALYSIS OF AMPHIBIAN HABITATS IN TALUKA BAKRANI, DISTRICT LARKANA, SINDH-PAKISTAN

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Amphibian are sensitive to the high values of physico-chemical parameters such as pH, electric conductivity (EC), total dissolved solids (TDS), total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl), sulphate (SO₄), phosphate (PO₄), nitrite (NO₂), nitrate (NO₃), carbon dioxide (CO₂) and potassium (K) which hinder growth and development of amphibians during initial phase of their life. Therefore, present study was proposed to evaluate water quality of amphibian habitats in Taluka Bakrani of District Larkana, wherefrom populations of three amphibian species including *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis* and *Bufo stomaticus* were confirmed in agricultural fields. Water sampling and physico-chemical analysis was conducted monthly for three years (2011-2013) using analytical instruments and standard procedures. The results showed values of all the parameters as followed: pH (7.8±0.7), EC (2559.4±1087.0), TDS (1759.6±677.1), T-Hard (584.6±229.9), T-Alk (312.8±72.8), Cl (447.9±120.7), SO₄ (475.6±162.0), PO₄ (413.4±93.1), NO₂ (5.7±3.5), NO₃ (8.6±4.0), CO₂ (19.0±4.0) and K (74.5±11.3). Value of all parameters (except pH and CO₂) was much higher than favorable limit that may be unsuitable especially for eggs and larvae of amphibian. This environmental instability implies the careless role of local people who may deteriorate the water quality of ponds by using chemicals like fertilizers, herbicides, insecticides and fungicides in high amounts. In this context, wildlife authorities must implement conservation rules in order to save amphibian fauna from being lost.

3. MARINE BIOLOGY

GENETIC DIFFERENTIATION AMONG MANGROVE CRABS BASED ON SDS-PAGE PROFILE

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The aim of the present study is to investigate the interrelationships among intertidal mangrove crab using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profile. Banding pattern of general proteins of *Ilyograpsus paludicola*, *Macrophthalmus depressus*, *M. pectinipes*, *M. laevis*, *M. sulcatus*, and *Grapsid* species were compared using Jaccard's similarity index (JSI). On the whole total 17 reproducible bands were used for analysis and genetic diversity was estimated based on the number of different protein peptides. JSI coefficients among species of genus *Macrophthalmus* ranged from 0.44 to 0.56, among species of genus *Macrophthalmus* and *Ilyograpsus paludicola* was 0.40 – 0.33 while the 0.166 – 0.222 between *Grapsid* species and *Ilyograpsus paludicola*. Analysis showed that *Ilyograpsus paludicola* closely related to *Macrophthalmus* than *grapsid* crabs.

ISOZYME VARIATION IN BANDING PATTERNS THROUGH CARBONATE DEHYDRATASE AND AMIDO BLACK IN THE MUSCLES OF SHRIMP SPECIES FOUND IN SONMIANI BAY, BALUCHISTAN, PAKISTAN

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Shrimps have been playing an economical role in farming and fishing industries all over the world. They are considered as white gold for the exporters in Pakistan. Due to their high exploitation many countries are trying to farm or raise them by semi intensive farming too, but due to lack of a condition factor of the species it's hard to manage. In Pakistan more than 17 species have been discovered belong to the Penaeidae. Biochemical variations of some enzymes were determined in five economically important species of Penaeidae collected from Sonmiani (*Fenneropenaeus indicus* (F1), *Fenneropenaeus merguensis* (F2), *Metapenaeus monoceros* (M1), *Metapenaeus affinis* (M2), and *Parapeneopsis stylifera* (P1)). The electrophoretic banding pattern carbonate dehydratase and amido black B10 were examined in five different tissues of shrimp after staining in comparison with SDS-PAGE results, F1 showed 5 bands and RM of each (0.041, 0.315, 0.466, 0.493, and 0.521), F2 revealed 7 bands with (0.033, 0.049, 0.066, 0.082, 0.557, 0.574, and 0.967) RM, M1 displayed 11 bands with (0.736, 0.694, 0.611, 0.556, 0.500, 0.444, 0.417, 0.347, 0.319, 0.292, and 0.236) relative mobility, M2 disclosed 3 banding patterns with (0.593, 0.712, and 0.966) RM and P1 showed 7 bands with RM (0.0274, 0.0411, 0.0685, 0.4247, 0.4384, 0.4521, and 0.4658). Bands were observed and compared with carbonate dehydratase and amido black B10. Few locus was found to be polymorphic in nature and a few were monomorphic. This study of Penaeidae is still in infancy, hence

this study holds enormous prospect in the development and application of molecular markers. Further studies with more molecular markers throughout their geographical ranges are highly recommended for the management of endangered inland wild populations which are commercially harvested for estimation of phenotypic relationships between species.

LENGTH-WEIGHT RELATIONSHIP OF *GERRES FILAMENTOSUS* FROM CLIFTON BEACH, KARACHI-PAKISTAN

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Gerres filamentosus is a marine fish, also reported from freshwater and brackish areas at a depth of 1m to 50m. Maximum total length is 35cm but common in 15cm. Dorsal fin having a long filament with IX spines and 10-11 soft rays. This is an Indo-Pacific fish. The present investigation concerns with the length-weight relationship of this species. The length-weight relationship is a significant tool in fisheries sciences. For this study, 249 specimens of *G. filamentosus* were collected from Clifton beach, Karachi. The results of present investigations show that *G. filamentosus* has a positive allometric growth type ($b > 3$). The value of correlation (r) between the length and weight of *G. filamentosus* was obtained > 0.80 , shows strong correlation between the length and weight.

SPATIAL, GENDER AND SIZE VARIABILITY IN METAL ACCUMULATION IN FIDDLER CRAB, (*UCA IRANICA*) FROM COASTAL ENVIRONMENT OF PAKISTAN

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The present study investigates the accumulation of copper, zinc, cobalt, lead and cadmium in tissues of the fiddler crab, *Uca iranica* (Pretzmann, 1971). Crab specimens were collected from two different environments, mangrove (Sandspit) and non-mangrove (Sonari) along the coast of Pakistan. The both sexes (male and female) were observed for monitoring purposes, then they categorized in two size classes (medium and large) according to the site specific size ranges of crab carapace. The results reveal that Cu, Zn and Co accumulations in tissues were observed significantly greater in non-mangrove area, while Pb and Cd levels were significantly higher in mangrove area. No significant intersexual differences were observed in Cu, Zn and Pb levels, however Co and Cd load were found significantly greater in tissues of female as compared to male crab. Linear regression analysis showed the significant linear correlation between the burden of Cu, Zn and Cd in tissues with the carapace size of the crab. The Cu, Zn and Pb concentrations were significantly greater in the sediment of mangrove habitat, whereas Co and Cd showed no difference between both sites. The levels of Cu and Zn in male crab reflected the habitat specific accumulation, but no correlation of any metal was found in tissues of female and metal levels in sediment. The results suggested that this species showed high variability with respect to spatial distribution, sexual and size for different heavy metal accumulation. It was observed that if this species will include the further monitoring program the both sexes treated separately, moreover, the male crab may be a more useful candidate as bioindicator.

ORGANOCHLORINES RESIDUES IN TISSUES OF *CERITHIDEOPSILLA CINGULATA* (GMELIN1791) (GASTROPODA: POTAMIDIDAE) IN MANGROVE STANDS AT SANDSPIT BACKWATER, KARACHI, PAKISTAN

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To assess pollution status of organochlorines and its impact on the abundance of *Cerithideopsisilla cingulata* in the mangroves region, sampling was done during August-December 2015, respectively in mangrove stands, at Sandspits backwater. During the sampling period *C. cingulata* were found in low concentration but number of specimen were more abundant. Pearson correlation showed that the *C. cingulata* numbers were inversely correlated to the total concentration of Σ OCPs and the number of specimen in the sediment were negatively correlated in terms of deregulated distribution of sedimentary OCPs. Our data will help to understand the organochlorine pollution status in mangrove sites preferred by *C. cingulata*. Collective tissues samples of *C. cingulata* were analyzed by employing the Matrix Solid Phase Dispersion (MSPD) and GC-ECD (Gas Chromatography with Electron Capture Detector). Concentration of polychlorinated biphenyls (PCBs), DDTs, chlordanes, BHCs, dieldrin, heptachlor epoxide, Aldrin, Dieldrin, Endosulfan, Dimethoate, Ethion, Fenitrothion, Heptachlor, and Chlorpyrifos Σ BHCs were the most predominant contaminants. The OCPs concentrations in mangrove stands at Sandspit backwater, Karachi were significantly in the range of a few to several $\mu\text{g g}^{-1}$ on a wet weight basis that is OCPs ranged from 0.004 $\mu\text{g g}^{-1}$ w.w with BHC (0.27 $\mu\text{g g}^{-1}$ w.w) and Heptachlor epoxide (0.200 $\mu\text{g g}^{-1}$ w.w). PCBs had an overall range 0.0072-0.277 $\mu\text{g g}^{-1}$ w.w where 2, 3', 4, 4', 5-Pentachlorobiphenyl and (PCB-115) and Decachlorobiphenyl (PCB-209) were the dominant components. The redox conditions and the decay processes which affect the organic matter, control the concentrations of PCBs and OCPs in *C. cingulata*. Results suggest that the variations in PCBs and OCPs content in *C. cingulata* largely due to digenetic processes rather than changes in pesticide input resulting from local human activities.

DISTRIBUTION OF OCTOLASMIS IN INFESTED CRABS, *PORTUNUS SANGUINOLENTUS* AND *P. PELAGICUS*

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Genus *Octolasmis* Gray, 1825 belong to subclass Cirripedia of class Crustacea. Of 2725 specimens of the two species of *Portunus* examined for the presence or absence of octolasmids, only 162 crabs (5.94%) were found infested. The number of octolasmids hosted by these crabs was 5356 and they belong to five species; namely *Octolasmis angulata* (Aurivillius, 1894), *O. cor* (Aurivillius, 1894), *O. lowei* (Darwin, 1851), *O. tridens* (Aurivillius, 1894) and *O. warwickii* Gray, 1825. Out of 5168 octolasmids, three were found attached either on fifty walking leg or on carapace. These octolasmids belong to species *O. warwickii*. Hence no specimen of *O. warwickii* was found inside the gill chamber. These octolasmids which were found inside the gill chambers

number 5165. Out of these, 4867 were found attached on the surface of the gills while 298 octolasmids (160 *O. lowei*, 136 *O. tridens* and 2 *O. angulata*) were found attached to the wall of the gill chambers and epipodites (gill cleaner). Right gill chambers harboured more octolasmids (2694 out of 5165 or 52.2%) than the left gill chamber (2471 out of 5165 or 47.8%). The average number of octolasmids found in the right and left gill chambers was 17.74 ± 37.22 SD and 16.26 ± 38.85 SD, respectively, which is not different significantly ($t=0.265$, $\alpha=0.05$). The occurrence and distribution of octolasmids on *Portunus pelagicus* and *P. sanguinolentus* has never been studied in detail from Pakistan.

THE PHYLOGENETIC RELATIONSHIP OF GENUS *CHARYBDIS* AND *THALAMITA* OF SUBFAMILY THALAMITINAE BASED ON CODING SEQUENCE CYTOCHROME OXIDASE (COI) FROM THE COASTAL WATERS OF PAKISTAN

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In this study, we determined the *Cytochrome oxidase* (COI) mitochondrial DNA gene from the genus *Charybdis* and *Thalamita* of subfamily **Thalamitinae** and elucidated phylogenetic relationships between representative species in the family Portunidae from the coastal waters of Pakistan. During the present study the molecular identification and confirmation of species was done by the amplification of mitochondrial DNA Cytochrome Oxidase COI gene. The procured DNA sequences were initially searched for sequence similarity using BLAST (Basic Local Alignment Search Tool), and the initial species confirmation based on at least 95%-100% identity for each query sequence. The partial coding sequence (CDs) of COI gene sequence, approximately, 700 base pairs. A total of 9 species identified, five from genus *Charybdis*: *C. feriata*, *C. helleri*, *C. annulata*, *C. lucifera* and four from genus *Thalamita*: *T. admete*, *T. savingyi*, *T. crenata* and *T. danae*. The nucleotide sequence translated into protein sequence for the confirmation of species status by the similarity and homology, which is defined in the international nucleotide database. The molecular phylogenetic analysis demonstrated that genus *Charybdis* is genetically closest to genus *and Charybdis* and *Thalamita* and monophyletic to family Portunidae.

USE OF SHELLFISH (CRABS AND SHRIMPS) AS A BIO-INDICATOR OF HEAVY METAL POLLUTION FROM COASTAL AREAS OF PAKISTAN

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In aquatic ecosystems, heavy metals are considered as the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. Heavy metals such as cadmium, chromium, copper, lead and zinc are of the most important pollutants that affect aquatic environment and shellfish. The objective of this study was to determine and compare the concentration of two essential (Zn and Cu), one non- Essential (Cr) and two toxic heavy metals (Pb and Cd) in the edible muscles of commercially important shellfish species (*Portunus reticulatus*, *P.*

segnis, *P. sanguinolentus*, *Scylla olivacea*, *Peanaeus monodon* and *P. indicus*) on gender and weight of each species. The bioaccumulation process of the elements based on Metal Pollution Index (MPI), evaluated as an attempt to use these organisms as bioindicators of heavy metals pollution. The results showed that significant variations ($p < 0.05$) of Zn, Cu, Pb, Cd, and Cr levels in edible muscles of shellfish were different with sex and weight of each investigated species. The present data also show that metal accumulation in shellfish are species specific and MPI factor of essential metals were higher than that of non-essential heavy metals. Therefore, it is suggested that shellfish species can be used as bioindicators or biomonitoring studies of metal pollution.

BENTHIC FORAMINIFERA ASSOCIATED WITH MARINE SPONGE *LIOSINA PARADOXA* AT SANDSPIT BACKWATER, KARACHI COAST

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Sponge harbors variety of mutualistic associated organisms that include diversified groups of organisms including micro and macrofauna and flora. Foraminifera are one of the important and diversified inhabitants of marine sponges and broadly comparable among meiofauna and macrofauna in mangroves. Sponge samples were taken from four quadrates (0.5 m²) of Sandspit backwater mangrove (*Avicennia marina*), Karachi coast from January to December 2013. These samples washed thoroughly with seawater and 200 ml volume retained in sample bottle. Duplicate samples preserved separately in 80% ethyl alcohol and 5% formalin solution. A total of eight genera were identified. Among these two genera were common namely *Peneroplis* spp. and *Bolivina* spp. with dominating genera *Peneroplis* spp. In Physicochemical parameters, temperature ranged between 21°C-32°C, whereas salinity and pH range was 35-42 psu and 6.8-7.6, respectively. Nutrients (NO₃⁻, NO₂⁻, NH₄⁺ and PO₄⁻ ions) were also influenced the diversity of foraminifera. Seasonal variation was observed during pre-monsoon (42%) and monsoon (20%) and post-monsoon (< 2%) season. Foraminifera has poorly investigated group from coastal waters of Pakistan. There is a dire need to gain attention for better understanding of their mutualistic role in aquatic food web specifically in mangrove ecosystem of Pakistan.

STUDIES ON DAY-NIGHT DISTRIBUTION, COMPOSITION AND ABUNDANCE OF COPEPOD GROUPS IN MANORA WATERS ALONG KARACHI COAST OF NORTH ARABIAN SEA, PAKISTAN

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The present study was conducted to determine the distribution, composition and abundance of copepods during night and day time from Manora waters during April 2008 to March 2009 and recorded three groups, Cyclopoid, Calanoid and Herpacticoid. Copepods are major parts of all

zooplankton groups. During the study period air temperature ranged from 19°C -27°C, water temperature ranged from 18°C to 28°C, Ph ranged from 7-7.5 and DO ranged from 4.5mg/ L⁻¹ to 9.3mg/ L⁻¹ during night time and in day time it was recorded as air temperature ranged from 20°C - 27°C, water temperature ranged from 19°C to 28°C, Ph ranged from 7-7.5 and DO ranged from 4.9mg/ L⁻¹ to 9.4mg/ L⁻¹ . During this study the cyclopoid comprising 25.09%, calanoid comprising 64.05% and herpacticoid comprising 10.84% in night time and 21.32%, 69.70% and 8.97% in day time, respectively. Copepods indicated monthly richness and evenness, ranging between 0.5-0.8 and 34-92 in day time and 0.5-1.0 and 11-99 in night, respectively. Density of copepods was positively correlated with air temperature, water temperature, salinity, Ph, Transparency and DO. The diversity indices such as Shannon's diversity index (1.039- 0.779 in night time and 0.912-0.706 in day time) were calculated. The calanoid among the copepod groups shows high abundance in night and day collection during the whole year 2008-9. This study reveals that copepod groups in manora waters are most abundant during day time and their abundance enhances the fishing activities during day time.

4. PALAEOLOGY

NEW FOSSIL REMAINS OF *HYOTHERIUM PILGRIMI* (MAMMALIA: CETARTIODACTYLA: SUIDAE), FROM MIDDLE MIOCENE ROCKS OF CHINJI FORMATION OF PAKISTAN

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The fossil remains of ancient extinct species of *Hyotherium pilgrimi* have been described from middle Miocene rocks of Chinji Formation of Siwaliks of Pakistan. Its described age is about 13 to 10 million years but its actual age may vary. It is closer to its smaller European relative *H. soemmeringi*. The studied material includes isolated premolars and molars. It is the ancestor of recent boar and domestic pig. This paper will provide new insight of anatomical morphology of *Hyotherium*.

LATE MIOCENE UNGULATE REMAINS FROM DHOK MORI MOTHAN (PADHRI), PAKISTAN

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The systematics of ungulate remains recovered from Dhok Mori Mothan (Padhri), Siwaliks of Pakistan has been investigated using morphological and biometric approaches. The identified material has been found belonging to two orders, two families, three genera and three species namely *Sivalhippus theobaldi*, (*Perissodactyla*, *Equidae*), *Selenoportax vexillarius*, (*Artiodactyla*, *Bovidae*-*Boselaphini*), and *Gazella lyddekeri*. (*Artiodactyla*, *Bovidae*-*Antilopini*). Biochronologic assessment of the ungulate assemblage reveals that the explored region is of Late Miocene (ca. 10–06 Ma) in age. The ecomorphic information of the studied taxa may portray an ecosystem depicting a change from closed/semi-closed vegetation to open vegetation, humid and warm climate to dry and seasonal one leading to the exploration of diverse environmental mosaics ranging from moist deciduous canopy forest to dry deciduous forest, open woodlands and savannahs. This study may contribute to the understanding of systematics of ungulate remains from Siwaliks of Pakistan.

TRUE UNGULATE REMAINS FROM LATE MIOCENE OF HASNOT, JHELUM

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The systematics of true ungulate remains collected from Hasnot area of Pakistani Siwaliks has been studied incorporating morphological and metrical methods. The specimens have been found belonging to two orders, four families and four species. This comprise *Sivalhippus*

perimense, (Perissodactyla, Equidae), *Selenoportax vexillarius*, (Artiodactyla, Bovidae-Boselaphini), *Hydaspitherium* sp. (Artiodactyla, Giraffidae), and *Hippopotamodon sivalens* (Artiodactyla, Suidae-Suinae). Biostratigraphic ranges of the identified specimens reveal that the studied area is of Late Miocene (ca. 10–06 Ma) in age. The findings of the study may depict the existence of semi-closed and open vegetation with seasonal climate leading to environmental changes from moist and dry deciduous canopy forest to open woodlands and savannahs during this time interval.

NEW FOSSIL REMAINS OF *KOBUS* (AN EXTINCT BOVID) FROM THE LATE PLIOCENE SIWALIK DEPOSITS OF PAKISTAN

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New dental remains of an extinct bovid species, *Kobus porrecticornis* from the late Pliocene of the Siwaliks of Punjab, Pakistan are described and systematic status is also discussed. This discovery will contribute to the understanding of evolution and phylogeny of living bovids. The discovered material include the lower teeth and exhibit the diagnostic characters of *Kobus* (Reduncini) including a distinct goat fold, a less sharp basal pillar, and a transverse flange at posterior side and less constricted lobes at labial side. On the basis of morphometric analysis and its comparison with previously published data these fossils are attributed to the species *Kobus porrecticornis*. This obligate grazer species attributes that swamps, grassland, Savanna woodland and even dry land having a source of water were the palaeoenvironmental conditions of the Siwaliks during late Pliocene.

COMPARATIVE ANALYSIS OF ENAMEL HYPOPLASIA BETWEEN SIWALIK REGION NEOGENE TRAGULIDS AND RHINOS

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Enamel Hypoplasia is a dental disorder that can be studied in recent as well as fossilized dentition. This defect is caused by depletion of highly sensitive cells named as ameloblasts. Production of these cells can be affected by nutritional, vegetational and ecological stress. Current study is on comparative analysis of enamel hypoplasia between Neogene tragulids and rhinos. Three extinct tragulid species; *Dorcatherium minus*, *Dorcatherium majus* and *Dorcabone anthracotherium* were studied and results showed that occurrence of enamel hypoplasia in these species is significantly high as compare with extinct rhinos. This difference for prevalence of enamel hypoplasia depicts that tragulids were facing more vegetational and predatory stresses than rhinos in Neogene habitats of Asian region.

**COMPARATIVE ANALYSIS OF ENVIRONMENTAL STRESS IN MIDDLE SIWALIK
HIPPARION BY USING ENAMEL HYPOPLASIA**

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Enamel Hypoplasia is a tooth malady that provides the permanent record of stresses during life history of an animal. As enamel is one of the hardest tissues of body so enamel hypoplasia can be a stress marker for environmental and physiological stresses faced by the extinct animals during their growth and development. Comparative analysis of three Siwalik *Hipparion* species, *H.theobaldi*, *H. nagriensis* and *H.antelopinum* has been carried out in this study. Result shows that all the three studied species present during 11.2- 3.5 Ma time span have high prevalence of single as well as multiple linear enamel hypoplasia on premolars and molars. The observed multiple linear enamel hypoplasia indicates periodic stress event in habitat of these extinct species. These results of enamel hypoplasia trace out the climatic and vegetational stress events that were responsible for extinction of all Siwalik representatives of *Hipparion* genus from the Asian region.

**SYSTEMATIC ACCOUNT OF NEW SIWALIK ARTIODACTYLE REMAINS FROM
THE MIDDLE MIOCENE OF PAKISTAN**

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New remains of five extinct artiodactyls genera from different localities of Chinji Formation of Pakistan have been described and discussed in this paper. The recovered material includes the upper and lower dentition as well as horn cores. The studied mammalian assemblage includes the genus *Dorcatherium* (Tragulidae), *Listriodon* (Suidae), *Giraffokeryx* (Giraffidae), *Miotragocerus* and *Gazella* (Bovidae). The comparative analysis of hypsodonty and dental morphometric properties of these herbivorous genera trace out the vegetational diversity of Middle Miocene. This study has provided the better understanding of the taxonomy, diversity, paleoecology and biogeography of Miocene fauna.

**COMPARATIVE STUDY OF FOSSIL PREMOLARS OF BOVIDS (MAMMALIA:
BOVIDAE) FROM SIWALIKS OF PAKISTAN**

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New dental elements comprising upper premolars of the bovids have been recovered from the four sites of the Siwaliks, namely Chinji, Dhok Pathan, Hasnot and Padri. The fossiliferous sites

are situated in the Punjab province, Pakistan and belong to the Lower and Middle Siwalik subgroups, ranging from Middle Miocene to Middle Pliocene in age (14.2 – 3.4 million years ago). On the basis of the comparative morphology and measurements, the material is assigned to *Gazella* sp., *Gazella lydekkeri*, *Elachistoceras kauristanensis*, *Tragoportax salmontanus*, *Tragoportax punjabicus*, *Selenoportax vexillarius*, *Selenoportax lydekkeri* and *Pachyportax latidens*. The fossils provide evidence for the existence of diverse Middle Miocene to Middle Pliocene bovid faunas in Pakistan. The comparative morphological and metric study of the different bovid species gives interesting information about the size variation within genera and intra-specific level. The Middle Miocene to Middle Pliocene of the Siwaliks was considerably presented mosaic palaeoenvironment.

LISTRIODON PENTAPOTAMIAE FROM DHOK BUN AMEER KHATOON, CHINJI FORMATION, PUNJAB, PAKISTAN

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The Middle Miocene sites of Dhok Bun Ameer Khatoon (11.2 - 14.2 Myr) have yielded copious remains of mammals. New remains of *Listriodon pentapotamiae* have been found and described in this article. The recovered material includes mandible fragments and isolated dentitions of *L. pentapotamae*. These specimens impart long awaited information on the distribution and systematic of the species. The aim of the study is to describe poorly documented Middle Miocene suids.

DIETARY PREFERENCES AND PALEOECOLOGICAL INTERPRETATIONS OF BOSELAPHINE (BOVIDAE) REMAINS FROM SIWALIKS OF PAKISTAN

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Boselaphine remains from Siwaliks of Pakistan provide a long and continuous record of diverse geochronologic ranges and ecological niches that in turn help to explore their paleoecology. The dietary preferences and paleoecology of four extinct species of boselaphines from Late Miocene of Potwar Siwaliks of Pakistan have been investigated incorporating microwear and hypsodonty methods. The species of Boselaphines included in this investigation comprise *Pachyportax latidens*, *Selenoportax vexillarius*, *Tragoportax* sp and *Miotragocerus gluten*. The microwear patterns of those species showed that *Pachyportax latidens* was grazing mixed feeder, *Selenoportax vexillarius* (MI= 1.03) had fruit and graze components in the diet. Medium sized *Tragoportax* sp. was feeding on browse diet before 8 Ma and incorporated fruit and graze in the diet after 8 Ma. *Miotragocerus gluten* feeder (MI= 1.38) was browsing mixed feeder. The hypsodonty analysis of the studied taxa revealed that there was a change in their dietary and habitat

adaptations from browsers/frugivorous to mixed feeders in closed and open habitats. Comparison of the results from these research tools suggest that the extinct boselaphines reflected the significant changes in dietary adaptations ranging from browsers to seasonal mixed feeders in closed and open habitats and showed affinities with grazers as well. The coexistence of studied boselaphine remains with their ungulate paleocommunity reveals the persistence of pronounced seasonal paleoclimate and intensification of South Asian Monsoon System during 8-6 Ma. The ever increasing seasonality induced the change in paleovegetation depicting the mosaics of diverse habitats ranging from tropical evergreen forest to subtropical ones, closed seasonal woodlands to wooded and grassy savannas.

**NEW REMAINS OF *BRACHYOTHERIUM PERIMENSE* FROM MIOCENE AND
PLIOCENE LOCALITIES OF THE SIWALIKS OF PAKISTAN**

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New dental material of *Brachyotherium perimense* has been recovered from Nagri and Dhok Pathan formations of the Siwaliks of Northern Pakistan. The dental remains include the deciduous as well as permanent upper and lower dentition. The comparative morphometric analysis has been used for the identification of specimens. The dental morphology of this species traces out the variation in vegetation of the Miocene and Pliocene period ecosystems of the Siwalik region. This species is an indicator of forested area habitat with soft diet and less humid conditions in the Nagri Formation.

**SOME NEW FOSSILS OF EVEN TOED MAMMALS FROM THE LATE
MIOCENE OF SIWALIKS**

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Fossil site Dhok Bun Ameer Khatoon and Lava yielded a significant amount of mammalian assemblage including four families of even-toed fossil mammal (Suidae, Tragulidae, Giraffidae, and Bovidae) of the Late Miocene. This site has well exposed Chinji and Nagri formation and has dated approximately 14.2Mya-9.5 Mya. This age confirms the diversity of different mammalian genera and is important Palaeoecologically, Palaeogeographically and Palaeoclimatologically. The main reason to conduct this study at these sites is to add additional information in the field of Paleontology. A detailed study is carried out on even-toed mammalian fossils found in Miocene rocks exposed at Dhok Bun Ameer Khatoon and Lava localities of Siwaliks. The described specimens are 15 which were well preserved and identified as even toed mammalian remains that are described in this thesis. Four specimens belong to *Giraffokeryx punjabiensis*, three specimens belongs to *Listriodon pentapotamiae*, one specimen belongs to *Dorcatherium majus*, five specimens belongs to *Gazella sp.* and one belongs to *Selenoportax Vexillarius*. The collection comprises isolated upper and lower teeth and fragments of mandibular ramii. Palaeoenvironmental data indicates that Miocene climate of Pakistan was most likely to be monsoonal as there is now a

days. All the feeding habits and distribution of these animals suggested the presence of abundant forests, herbaceous, grassy and bushy vegetation in the Dhok Bun Ameer Khatoon and its surroundings.

**NEW REMAINS OF MIDDLE MIOCENE GIRAFFIDS FROM
DISTRICT CHAKWAL, PAKISTAN**

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Eight new giraffid remains described in this paper comprising isolated upper and lower molars, premolars and a fragment of mandible are recovered during field work from the middle Miocene localities like Dhok Bun Amir Khatoon, Chinji Rest house and Parrehwala of Lower Siwaliks, District Chakwal, Pakistan. Four specimens belong to species *Giraffokeryx punjabiensis* and four specimens belong to the species *Giraffa Priscilla* which has relatively more broad and brachydont teeth, major cusps not in straight line and more prominent styles/stylids, anterior rib and metastyle as compared to *Giraffokeryx punjabiensis*.

5. WILDLIFE, DIVERSITY AND CONSERVATION

POPULATION DENSITY AND HABITAT ASSOCIATION OF CHUKAR PARTRIDGE (*ALECTORIS CHUKAR*) IN KALA CHITTA NATIONAL PARK, DISTRICT ATTOCK

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Chukar partridge (*Alectoris chukar*) is a medium-sized game bird belonging to family Phasianidae. Population of Chukar Partridge has declined over the time mainly due to habitat fragmentation and excessive hunting. Study on population density and habitat association of Chukar Partridge was conducted from November 2015 to June 2016 in Kala Chitta National Park, having an area of 36,965 ha, located at 33°42'50" N and 72°10'27" E. For this purpose, study area was delineated into three habitat types i.e., i) Mountainous forest and associated grassland ii) Open cultivated land iii) Wetland and associated natural vegetation. In each habitat type, three fixed transects having length of 200 m and width varying from 10 m to 50 m, were laid down. For habitat analysis, vegetative survey of selected study sites was conducted by using quadrat method by taking 90 quadrates. Overall population density of chukar partridge in study area was estimated at 2.34 birds / ha while it was 6.41 birds/ha in mountainous forest & associated grassland and 0.62 birds/ha in open cultivated land habitat. Fifty-two plant species were recorded from habitat of Chukar Partridge, out of which 11 were trees, 10 shrubs, 24 herbs and seven grasses. Most preferred habitat was mountainous forest and associated grass land dominated by *Dodonea viscosa*, *Carissa opaca*, *Olea ferruginea*, *Acacia modesta*, *Acacia nilotica* and *Adiantum incisum*. Other important features of habitat were rocky mountainous ridges, natural springs and dense vegetation. Chukar Partridge was not found in wetlands and associated natural vegetation habitat probably due to high disturbance and scarce vegetation.

ESOPHAGATOMY IN HOUBARA BUSTARD (*CHLAMYDOTIS MACQUEENII*) AT SIR BANI YAS ISLAND, UNITED ARAB EMIRATES

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The Houbara Bustard (*Chlamydotis macqueenii*) is a medium-sized gruiform of the family Otididae which inhabits semi-desert and shrub-covered arid plains of West Asia and North Africa. In recent years the species has seen a severe decline throughout its habitat and is vulnerable according to IUCN, 2016. The purpose of this study was to investigate the abscess formation on neck region and resolve the surgical treatment complications so that it plays a positive role in the treatment and conservation of Houbara Bustard. One and a half year old two males Houbara Bustard were treated at Sir Bani Yas Island veterinary hospital for the evaluation of abscess on lateral side of neck just above the trachea and pre-crop esophagus. They were examined several times but no abnormalities were observed except abscess on the neck, which was very hard and

subcutaneous on palpation. Sample was aspirated from this abscess and sent to the laboratory for bacteriological analysis; reports indicated the presence of *Methylobacterium mesophilicum* and *E. coli*. The birds were admitted to hospital to keep under strict observation. Houbara Bustard were feeding properly and no obvious abnormality was observed. Antibiotics treatment was applied but abscess prevailed. One week later surgical removal of abscess was planned, these birds were anesthetized. There are no reports for local anesthesia in bustards but a 2mg/bird intramuscular dose of Ketamine hydrochloride (100mg/ml) was experimented that proved to be useful. Esophageal tube was passed down to the esophagus to avoid any damage. On superficial incision a beetle spp. was found instead of abscess with its tarsal claws stuck in esophagus. Pre-crop esophagus was necrotized, fragile and adhesions were found. Beetle was taken out and pre-crop esophagus was closed with absorbable sutures. Esophageal tube remained during the whole procedure to avoid the closure of esophagus. A day after surgery, birds were active and were fed with powder feed. No abnormal behavior was observed. They were released successfully to the enclosure after 22 days of extensive care.

**SEASONAL VARIATIONS IN THE DIET OF ENDEMIC FROG
NANORANA VICINA (DICROGLOSSIDAE)**

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The Murree Hills Frog (*Nanorana vicina*) holds high conservation significance, for it is endemic to South-east Asian highlands. The data on ecology and biology of Murree Hills Frog are deficient in Pakistan. The present study was designed to study the diet of the frog during breeding and non-breeding seasons. The surveys were carried out in the selected sites of Murree and Kashmir during breeding season (May-September, 2015-2016) and non-breeding season (October-April, 2015-2016). The specimens were captured using dip nets, and standard stomach content flushing technique was used to document the diet. The food items were identified under stereo-microscope to the lowest possible taxonomic level (vegetation to species while insects to order level). Stomach content analysis of 20 frogs revealed a total of twelve food items (plant species and insect orders) during breeding season. Of which, nine were of animal origin (insect orders) and two were plant species. The most frequent food item belonged to order Coleoptera (% frequency) of 33.13 followed by Hymenoptera (16.47), Orthoptera (13.04), Dermaptera (11.33), Hemiptera (9.17), Isoptera (6.16), Diptera (5) and Lepidoptera (2.25). The frequency (%) of *Trifolium repens*, *Cynodon dactylon*, unidentified bones and unidentified animal body parts were 1.5, 1.26, 1.2 and 1.72, respectively. The food items from order Coleoptera contributed the most in the volume (ml) 1.3, followed by Diptera 0.47, Dermaptera 0.29, Hymenoptera 0.17, Hemiptera 0.14, unidentified bones 0.13, unidentified animals 0.10, Isoptera 0.08, *Trifolium repens* 0.042, Orthoptera 0.036, *Cyanodon dactylon* 0.02 and Lepidoptera 0.01. While the analysis of stomach contents of 20 frogs during non-breeding season showed a total of ten food items. Of which, eight were insect orders. The most frequent (%) food item was from insect order Coleoptera of 26.37, followed by Diptera (16.01), Orthoptera (13.04), Hemiptera (12.0), Homoptera (8.5), Lepidoptera (4.28), unidentified material (1.295), Isoptera (1.25) and unidentified bones (0.96). The frequency of Homoptera, Lepidoptera, unidentified material, Isoptera and unidentified bones were 8.5, 4.28, 1.295, 1.25 and 0.96, respectively. The food items belonging to Hymenoptera contributed the

most in the volume (ml) 0.49 followed by Coleoptera 0.40, Orthoptera 0.24, Dermaptera 0.15, Hemiptera 0.15, Bones 0.13, Lepidoptera 0.10, Unidentified 0.07, Isoptera 0.05 and Diptera 0.05. It is concluded that Muree Hills Frog is mainly insectivorous. However, opportunistically it also feeds on other small frogs and vegetation. The food item taxa during breeding and non-breeding seasons did not differ significantly ($t = -0.18$; $df = 19$; $P = 0.85$). The similarity index showed 81 % similarity between food item taxa during the two seasons. Few plant species such as *Cyanodon dactylon* and *Trifolium repens* and specimens of insect order Dermaptera were only recorded during breeding seasons while specimens of insect order Homoptera were recorded during non-breeding season. It is suggested that the association of habitat type, particularly vegetation of the area, with the insects reported during the study as food items of the frog be carried out to understand ecological role of the species, and to design conservation strategy for area in future.

ANALYSIS OF DIET DEFECATION SITE OF BLACK ROCK AGAMA (*LAUDAKIA MELANURA*) IN MORI SAID ALI GAME RESERVE, AJ&K.

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The Black Rock Agama (*Laudakia melanura*) in Pakistan is widely distributed in areas of Salt Range, North Punjab, Waziristan, Khyber Pakhtunkhwa and Kashmir. The present study was carried out to investigate the diet and to record the characteristics of defecation sites of the lizard in Mori Said Ali Game Reserve AJ&K. We studied diet of the lizard through stomach content flushing of 24 specimens, and fecal analysis of 22 composite samples, containing 10 pellets each. The defecation site characteristics were recorded by marking 3-4 defecation sites in each habitat. Four transects each with a length of 100m and width of 30m were laid out in each sampling site to gather data on the substrate, cover objects, distance to the nearest wetland and defecations sites. We recorded 18 food item taxa from the diet of Black Rock Agama using fecal analysis. This included nine species of plants (50%) and nine insect orders (50%). The most frequent plant species was *Pyrus pashia*, *Cyanodon dactylon*, *Rumex hastatus*. The least frequent plant species included *Trifolium repens* and *Isodon rugosus*. The majority of insect taxa recovered from the fecal samples of the lizards belonged to order Coleoptera followed by Hymenoptera and Hemiptera. A total of 15 food item taxa were recorded from the diet of Black Rock Agama using stomach content analysis. This includes eight species (53%) of plants and seven insect orders (47%). The most frequent plant species was *Isodon rugosus* followed by *Pyrus pashia*, *Oenothera rosea* and *Rumex hastatus* while the least frequent plant species included *Cynodon dactylon* and *Tagetes minuta*. The majority of insect taxa recovered from the fecal samples of the lizards belonged to order Coleoptera followed by Hymenoptera and Hemiptera. The least common insect taxa belong to the order Orthoptera and Isoptera. The similarity index showed that the similarity in food item taxa using fecal sample analysis at sites rocky habitat-human habitations and rocky-stream habitat was highest (38% each) followed by human habitations-stream habitat (33%), rocky habitat-cropland habitat (30%), cropland habitat-human habitations and cropland habitat-stream habitat (24% each) whereas the similarity in food item taxa using stomach content analysis at sites rocky habitat-cropland habitat was highest (87%) followed by stream habitat-rocky habitat and stream habitat-cropland habitat (66% each), rocky habitat-human habitat (25% each). The Kruskal-Wallis test reveal that the number of food item taxa in the four sites did not differ

significantly using fecal analysis ($P=0.12$) and stomach content analysis ($P=0.36$). The number of food item taxa differed did not differ significantly in the two methods ($P> 0.05$). However, a herb *Veronica persica* and insect orders Diptera and Hemiptera were recorded only in the fecal analysis and a herb *Tagetes minuta* was recorded in the stomach contents only. The defecation site characteristics were recorded by marking 3-4 defecation sites in each habitat. Four transects each with a length of 100m and width of 30m were laid out in each sampling site to gather data on the substrate, cover objects, distance to the nearest wetland and defecations sites. In the fecal analysis nine plant species were recorded and different orders. The dominant food specie was *Pyrus pashia* (74%, 87.5%, 88% and 73% respectively) among the four sites and the order Coleoptera and Orthoptera. In addition to *Pyrus pashia*, *Isodon rugosus*, *Berberius lycuim* and *Rumex hastatus* were also recorded. Herbs species from feacal samples consists of *Oenothera rosea*, *Trifolium repens* and *Sausseria*. In stomach flushing different other species were found which were not present in feacal samples which included *Tagetes minuta*. The defecation site characteristics were characterised by rocky substrate. The defecation site had high herb and shrub species richness (number of species= 10) followed by four shrub species. The most frequent herb included *Trifolium repens* and the most frequent shrub was *Pyrus pashia*.

POPULATION SIZE AND HABITAT OF INDIAN GAZELLE (*GAZELLA BENNETTII*) IN NIZAMPUR AREA, DISTRICT NOWSHEHRA PAKISTAN

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The Indian gazelle (*Gazella bennettii*) also known as Chinkara, is a very adaptable wild animal. Present study determined the population size and habitat association of Indian gazelle in Manglot near Nizampur area of District Nowshera from September 2015 to July 2016. The data on population density was collected by direct counting using vantage point method and scanning from these vantage points. A total of 19 Indian gazelles were observed at four different study sites located between 328 m and 504 m elevation in the study area. Of the total individuals observed, 21% were males, 37% were females, 32% were yearlings and 10% were young. Habitat analysis was done through vegetation survey using quadrat method in its habitat. Relative density, relative frequency, relative cover and importance value index were calculated for all plant species recorded in Indian gazelle habitat at four study sites. A total of 22 quadrates were taken between the elevation of 328 m and 504 m near selected Vantage Points. As many as 33 plant species belonging to 16 families were recorded in Indian gazelle habitat among which trees were 24.2%, shrubs 30.3%, herbs 18.2% and grasses 27.3%. Indian gazelle in the study area is facing problems due to increasing human population and also extension of agriculture practices in its potential habitat.

POPULATION DENSITY AND HABITAT ASSOCIATION OF GREY FRANCOLIN (*FRANCOLINUS PONDICERENUS*) IN TANDA DAM WILDLIFE PARK, DISTRICT KOHAT

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Grey Francolin is a medium-sized game bird which is considered friend of the farmers as it is believed to consume insects which are harmful to crops, hence, serving as biological controlling agent.

Reportedly, population of Grey Francolin has declined over the time in Pakistan mainly due to habitat destruction and excessive hunting. Population density and habitat association of Grey Francolin (*Francolinus pondicerenus*) was determined in Tanda Dam Wildlife Park from November, 2015 to June, 2016. The Park was established in 1989 over an area of 1133 ha, altitude ranges from 520m to 750m and has been declared Ramsar site as it provides habitat to several migratory water fowl species in winter. For this purpose, study area was stratified in to three different habitat types i.e. Sub-Tropical Board Leaved Evergreen Forest, Open Grass Land and Riparian Zone. Data on population of Grey Francolin was collected through direct observations using line transect method. In each habitat type three transects were laid down of 200 m long and 20 m wide each. For habitat association, vegetation survey of study area was conducted in three selected habitat types using quadrat methods taking 90 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 Francolin. Population density of Grey Francolin recorded in all three habitats was 4.62 ± 0.65 birds/Km². Sub-Tropical Board Leaved Evergreen Forest habitat has population density of 2.75 ± 0.81 birds/Km², Open Grass Land 2.0 ± 0.44 birds/Km² and Riparian Zone 1.25 ± 0.25 birds/Km². Population densities were significantly different among habitats ($p = 0.03$, $d.f = 2$ at $\alpha = 0.05$). Collectively, 38 plant species were recorded from the habitat of Grey Francolin in the study containing 9 trees, 10 shrubs, 10 herbs and 9 grasses. Most preferred habitat of Grey Francolin in the study area was Sub-Tropical Broad Leaved Evergreen Forest dominated by shrubs having higher population density 2.75 ± 0.81 birds/Km².

FOOD PREFERENCE OF RHESUS MONKEY (*MACACA MULATTA*) IN THE MARGALLA HILLS NATIONAL PARK, ISLAMABAD

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Study on food preference of Rhesus monkey (*Macaca mulatta*) was conducted in the Margalla Hills National Park (MHNP) Islamabad which falls in Murree foothills between 450m--1500m elevation. Data on foraging was collected by direct observations on five selected groups of rhesus monkey which were classified into five age/sex classes such as adult males, adult females, sub adults, juveniles and infants. Seven feeding categories: arboreal, ground, provisioning, scavenging, begging, stealing, snatching and suckling were defined. A total of 540 observations were taken for all age and sex classes. The arboreal: 336 (62.3%) and ground feeding: 206 (38.1%) were widely used by the monkeys than stealing: 53 (9.8%) and snatching: 47 (8.7%). Food composition was determined on the basis of field observations and fecal analysis. Rhesus monkey consumed 30 plant species including leaves, fruit, flowers, buds, seeds, petioles and shoot juice. A total 150 fecal pellets were collected and subjected to both macroscopic and micro-histological analysis. The macroscopic fecal analysis showed presence of indigestible hard parts including seeds: *Psidium guajava* (8.67%), *Citrus sinensis* (5.34%), *Punica granatum* (2.67%), *Solanum lycopersicum* (04%), *Pyrus pashia* (02%), *Cassia fistula* (12%), *Ficus spp.* (7.34%); seed coats: *Arachis hypogaea* (10.67%), *Solanum lycopersicum* (04%), *Cassia fistula* (03%), *Vigna radiate* (09%); blades of forbs: *Elusine indica* (32%), *Cynodon dactylon* (22.67%), *Asparagus officinalis* (2.6%) and pebbles (18%). Results showed that the monkeys exploited restricted food items during autumn and winter. Plant species identified through micro-histological analysis for spring and summer were 25 as compared to 19 in autumn and winter. Presence of cells and cell fragments of *Carissa opaca* 129 (79%), *Cassia fistula* 24 (16%), *Cynodon dactylon* 34 (22.67%), *Dalbergia sissoo* 113 (75.34%) and *Ficus bengalensis* 63 (42%) in the slides showed their relative importance in autumn and winter diets. Presence of cells and

cell fragments of *Carissa opaca* 136 (90.67%), *Dalbergia sissoo* 129 (86%) and *Ficus bengalensis* 89 (59.34%), *Elusine indica* 63 (42%) *Buxus papilosa* 52 (34.67%) *Ficus religiosa* 37 (24.67%), *Acacia nilotica* 34 (22.67%), *Cynodon dactylon* 34 (22.67%), *Asparagus officinalis* 27 (18%) and *Cassia fistula* 46(30%) in the slides showed their relative importance in the seasonal diets. Seasonal variations were linked to vegetation structure of the locality and abundance of seasonal food plants. The preferred plant species were *Buxus papilosa*, *Carissa opaca*, *Cassia fistula*, *Cynodon dactylon*, *Dalbergia sissoo*, *Elusine indica* and *Ficus bengalensis*. Overall food composition of Rhesus monkey in study area consisted of 83 percent plants diet, 14 percent provisioned food and 03 percent scavenged diet.

COMPARITIVE ENDOPARASITIC ANALYSIS AT SELECTED CAPTIVE LOCALITIES OF ANTILOPE *CERVICAPRA* 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 deworming but we were not sure that each individual in the herd was taking proper dose of dewormer. There is need to revise the de-worming protocol otherwise already threatened animal would face more troubles and to spend healthy life. It is recommended antelopes kept at Lal Sohanra National Park should also be given proper dewormer along with natural wormicides

HUMAN-COMMON LEOPARD (*PANTHERA PARDUS*) CONFLICT IN DISTRICT KOTLI, AZAD JAMMU & KASHMIR

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Human-wildlife conflict is a serious issue at global level. It caused the extirpation of many carnivore species from their earlier ranges. Present study aimed to quantify the livestock depredation

by Common Leopard (*Panthera pardus*) in the District Kotli, Azad Jammu and Kashmir from April 2015 to March 2016. Questionnaire method was used to assess the depredation from 8 study sites of the study area. A total of 241 livestock depredation events were recorded during the study period. Livestock depredation fluctuated in different months of the year. Highest depredation was recorded in July (n=48, 19.91%) and lowest in October (n=8, 3.31%). Goats (53.94%) and sheep (21.57%) were more vulnerable to depredation as compared to cows (7.05%). Depredation event occurred at night were higher (59.75%) as compared to the day time (8.71%). Local community beard high economic loss (PKR= 1.547 million) that resulted in the building of their negative attitude (89%) toward Common leopard presence and want to eliminate the species from their surroundings. Increasing human population, decreased leopard's natural prey, and poor hardening practices are considered to be the main factors of Human-Leopard conflict. This study provides baseline data for future conservation management activities of this vulnerable animal in study area.

POPULATION ESTIMATION AND HABITAT ANALYSIS OF SMOOTH COATED OTTERS (*LUTROGALE PERSPICILLATA SINDICA*) IN NARA CANAL, NARA, DISTRICT KHAIRPUR MIR'S, SINDH, PAKISTAN

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The present study was designed to estimate the population size and habitat of Smooth coated Otters (*Lutrogle perspicillata sindica*) in Nara Canal Nara, District Khairpur Mir's Sindh, Pakistan, during May –December 2016 with the objectives to confirm the existence and to identify various threats to Otter population in Nara Canal. An area of about 130 Km was traversed covering 25 different sites of Otter existence. The existence of Otter was confirmed 22 sites in Nara Canal. Evidence like Otter tracks, remains of fish eaten by Otter, Otter foot prints, gathering of holts and interviewed were conducted by local residents and fishermen to verify the Otter existence at every site. Nine sites were confirmed, where an Otter population exists throughout the year, whereas all the other sites are occasionally visited by Otters over different seasons, in search of food. Various threats to the species identified during the surveys, including habitat degradation, hunting for fur, water pollution, weak enforcement of Wild Life laws and competition and conflicts between Otters and fishermen. The total population of Smooth Coated Otter was also estimated over the 22 sites as 68. Overall results indicate that the Otter population in all over the observed sites was not encouraging. Therefore the strict watch and ward and habitat improvement with the co-operation Forest Department will be helpful to conserve this species.

FECAL MATTER AS A BIOINDICATOR OF POLLUTION IN PUNJAB URIAL (OVIS ORIENTALIS PUNJABIENSIS) HOUSED AT BAHAWALPUR AND LAHORE ZOO

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The Punjab urial is an endemic sub species of Pakistan and is protected under all provincial laws. It is known to have a restricted distribution range and found only in the Salt and Kala Chitta

Mountain ranges. For the conservation purposes it is also kept in captivity. In the present study feces of Punjab Uril (*Ovis orientalis punjabiensis*) used as bioindicator of heavy metal contamination. The samples of feces were collected along with water, feed and soil from Bahawalpur and Lahore Zoo. After chemical digestion the samples were run for Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). The following metals zinc (Zn), Lead (Pb), Cadmium (Cd) and Copper (Cu) were studied. The amount of Zinc and Copper were observed higher in fecal samples such as 4.88 ± 0.000944 and 0.118 ± 0.001 for Bahawalpur Zoo and 5.33 ± 0.030 and 0.135 ± 0.00054 for Lahore Zoo as compared to other samples in both Bahawalpur and Lahore Zoo. The amount of Cadmium and Lead was found to be lower in both Bahawalpur and Lahore Zoo as the values for Bahawalpur Zoo were 0.020 ± 0.008 and 0.036 ± 0.00094 and values for Lahore Zoo were 0.00733 ± 0.000545 and 0.0293 ± 0.001 respectively. Analysis of fecal material along with feed, water and soil showed effects of air pollution due to heavy traffic on the roads near the Zoos.

POPULATION DENSITY AND HABITAT OF CAPE HARE (*LEPUS CAPENSIS*) IN SHIGAR VALLEY, CENTRAL KARAKORUM NATIONAL PARK – PAKISTAN

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The study was designed to determine the population density and habitat association of cape hare (*Lepus capensis*) in Shigar Valley, Central Karakorum National Park (SVCKNP) through distance sampling by using line transect census method in July 2015 to March 2016. Population density and habitat association of cape hares were studied twice a year, during winter (November to march) and in summer (June to august) by dividing the SVCKNP into four potential sampling sites having (herbs and shrub land, open grass/arable land, forest/open land, forest/arable with sand dunes and rock terrain). A total of twenty four transects were carried out in all selected sites. The population density of cape hare at SVCKNP was 3.94 individuals ha⁻¹ (95% CI). The encounter rate of cape hare was found variable in different habitat types of CKNP. Maximum encounter rate was recorded in open grass/arable land (93.2%) followed by forest/open land having agricultural boundaries (87.7%), herb and shrub land (50.6%) and very less in forest/open land having sand dunes and rock terrain (31.6%). A total of fifty-nine plants species were recorded at SVCKNP that are associated with habitat of cape hare. The preferred habitat for cape hare at SVCKNP was an area having a thick cover of herbs and shrubs followed by open forest land with agricultural boundaries and very less associated with trees.

POPULATION DENSITY AND HABITAT PREFERENCES OF RED FOX (*VULPUS VULPUS*) IN CHAKWAL, PAKISTAN

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The red fox (*Vulpus vulpus*) is a carnivore that is classified as least concern according to The IUCN Red List of Threatened Species (2016). However, in Pakistan red fox is considered as

near threatened, due to continuous habitat destruction and depletion of food resources. The objective of the study was to identify habitat preferences and population density of red fox in Chakwal, Pakistan. Line transect census method was used to estimate the population density of red fox through direct sighting and indirect method of burrow counting, presence of foot prints and scats. A total of 30 transects were carried out at three potential sites (Devi, Potaki and Chumbi surla) in Chakwal based on preliminary survey. Habitat preference was estimated by comparing three different study sites and found that Devi area is preferred habitat for red fox. A total of 41 plant species were recorded in the study areas, among them *Cynodon dactylon* is major herb found to provide shelter to red fox in all potential study sites based on Importance Value Index (I.V.I) at Chumbi surla (IVI=208.8) followed by Devi (IVI=185.93) and Potaki (IVI=142.33). The maximum population density of red fox through direct sighting in Devi site was 0.03 individual/hectares compared to Potaki and Chumbi surla having 0.01 individual/hectares. The indirect estimation method revealed that maximum dens were found in Potaki area compared to Devi and Chumbi Surla, while foot print and scats were found maximum in Devi area compared to other study sites. It is concluded that red fox preferred habitat is Devi area in Chakwal, Pakistan

CONSERVATION STATUS OF AMPHIBIANS IN WILD ENVIRONMENT OF DISTRICT JAMSHORO, SINDH-PAKISTAN

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Present study was conducted in District Jamshoro (11,517 Km²) for the determination of water quality of amphibian habitations in agricultural ponds for the period of three years i.e. 2011-2013. Investigation comprised of regular surveys of permanent amphibian habitations (18) for the collection of water samples and laboratory analytical study was also carried out for the evaluation of some main physico-chemical parameters (pH, EC, TDS, T-Hard, T-Alk, Cl, SO₄, PO₄, NO₂, NO₃, K and CO₂) as these parameters are greatly responsible for making water clean or polluted on the basis of their concentration. Specific scientific methodology and distinct analytical instruments were applied discretely for the evaluation of each parameter. Results of this study showed normal value of pH and CO₂; however concentration of other parameters was very high. Value of pH (6.5-9.4), EC $\mu\text{S cm}^{-1}$ (945.8-5130.0), TDS mg L⁻¹ (580.6-3437.1), T-Hard mg L⁻¹ (130.0-950.9), T-Alk mg L⁻¹ (145.5-537.0), Cl mg L⁻¹ (177.5-685.15), SO₄ mg L⁻¹ (200.0-800.0), PO₄ mg L⁻¹ (150.8-800.0), NO₂ mg L⁻¹, (0.1-14.6), NO₃ mg L⁻¹ (1.0-15.75), CO₂ mg L⁻¹ (12.0-26.0) and K mg L⁻¹ (46.8-105.7) was inauspicious for the amphibians of the study area.

MONITORING THE CAPTIVE BEHAVIOR AND ACTIVITY PATTERN OF OSTRICH AT UVAS

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A study was conducted on behaviour of ostrich in captivity at UVAS, Ravi campus, Pattoki. There are three young ostriches housed at the Captive Breeding and research centre in the Department

of Wildlife and Ecology at UVAS. The behavior of the captive ratites was documented by direct observation with naked eye during September and October. Observations were made at two times throughout the day i.e. morning and evening. The monitoring duration was one hour and time spent in various activities like walking, running, jumping, feeding, drinking, pecking, excretion etc. was recorded in seconds. It was noted that most time was spent in walking (14%) while least time was spent in drinking (2%). The activities were performed in the following order of frequency; Walking (14%)>Standing(8.75%)>Pecking (5.55%)>Feeding (5%)> Running (2.5%) > Drinking(2%). It was also observed that some activities were more frequently performed during the morning time such as standing while others were performed more often during the evening such as feeding, walking and running. The pecking rate was very high all the time.

OSTRICH: THE MELLENIUM BIRD FOR MEAT AND BUSINESS

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Ostrich is the world's largest Ratite (flightless birds without keel bone) bird, 2.75m in height and 150kg in mass and its feathers are fluffy and symmetrical. The adult male bird has a grey colored neck with black and white wing primaries and tail feathers. The female has white to light grey wing primaries and tail feathers with dull brown to grey color all over the body. Young chicks are mottled brown, yellow, cream and orange with black quills on back while Juvenile birds resemble the females. The head is 1.8-2.75m above the ground due to combination of large legs. The eyes are 50mm in diameter. Ostrich is the largest vertebrate and has ability to position his head to produce an image from in front of and below the eye. The large blind spots on above and behind the head are considered to shade the eye. Ostrich spends all the time walking around its environment and unable to fly and can only run in case of any danger attaining a speed of 60-70km/h. The large amount of feed energy is saved in the tendons which are used by the ostrich for running. Ostrich is a digitigrades, but uniquely, it has only two toes. The ostrich has no substantial pectoral muscles and wings are poorly developed. Though ostrich can't fly, the presence of air sacs, pneumatized bones, strong pygostyle and presence of some wing bones give evidence that ostrich is evolved from flying ancestors. Ostrich is an herbivore bird, mostly eats green grass. Puberty starts at 3 years but females mature earlier. Female can lay eggs for 30-40 years. That is a reason for fast increase in ostrich farming. The demand for breeder is also increasing worldwide. First commercial farm of ostrich was introduced in South Africa in 1863. Now this business has spread to about more than 100 countries. Some of the reasons for ostrich farming includes as it lays a good quality and heavy eggs. They can tolerate from -1 to 50 centigrade. Environment of Pakistan is quite good for successful ostrich farming.

GASTRIC IMPACTION IN GROWING OSTRICH

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The ostrich as an alternative livestock business venture has been recognized worldwide. The main purpose of ostrich farming is meat production but many other useful

products like skin and feathers are also obtained. Good husbandry services and sound managerial practices are required to raise ostrich chicks successfully. It is inevitable to encounter many diseases of ostriches as a consequence of the rapid expansion of ostrich farming. In commercial ostrich farming, gastric diseases are hydra head problem and to measure and understand the scale of this disease is really hard nut to crack, which is common reason of increased mortality and economic losses. Among gastric diseases, gastric impaction is more frequent and is characterized by anorexia, cachexia, dehydration, apathy, decubitus and dry and reduced faeces. Impaction may also be acute or chronic in nature caused by improper management, stress, consumption of non-feeding material (stone, sand, wood pieces, plastic, glass, and metallic objects) as well as enteritis due to infectious bacteria *i.e.* *Escherichia coli*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Salmonella* and *Clostridium* and improper diet. Usually, palpation of abdomen, radiography techniques is practiced to diagnose the gastric impaction problems. Generally, impaction is treated with mineral oil, magnesium sulphate and psyllium and surgery is the last option in acute cases.

**EFFECT OF DIFFERENT FEEDING STRATEGIES ON THE
PRODUCTION PERFORMANCE OF BUDGERIGARS
(*MELOPSITACUS UNDULATES*)**

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Eighteen pairs of adult budgerigars (*Melopsitacus undulates*) were purchased from local market, weighed individually and were randomly divided into three experimental groups having three replicates (each) and each replicate had two pairs of budgerigars. Ten different types of seeds namely bajra, cheena, yellow kangini, red kangini, sunflower seeds, safflower seeds, rice, chary available in local market were offered to the budgerigars during acclimatization period of ten days to find out the preferred seeds as pre-experimental trial. The experimental birds were fed on 100% millet mixed with different levels of vitamin (vitasolTM Super) *i.e.* 0.25g and 0.75g. It was observed that feed consumed by the experimental budgerigars varied statistically significantly in various months ($p < 0.01$). Maximum feed was consumed by the birds in November (7.05 ± 0.19). Feed consumed by the birds during October (6.73 ± 0.17) and September (6.78 ± 0.11) was also statistical similar to that of November, 2008. Mean feed consumed by the experimental groups did not show any statistical variation. Maximum number of eggs were laid during August (2.78 ± 0.33) and minimum during July (0.00 ± 0.00). This variation was statistically different. Maximum eggs were laid by the birds served with millet +0.75g vitasolTM Super (0.23 ± 0.33). Mean maximum hatchability rate was also shown by the group which was served with millet +0.75g vitasolTM Super (1.23 ± 2.0). Maximum final weight and gain in weight was statistically higher in females (38.76 ± 0.78 ; 7.10 ± 1.06 , respectively).

NEW RECORD OF GENUS *OPISTHORCHIS* BLANCHARD, 1895 IN BLACK CROWNED NIGHT HERON *NYCTICORAX NYCTICORAX* FROM HAMAL LAKE, SINDH, PAKISTAN

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Six Black Crowned Night Heron *Nycticorax nycticorax* were captured alive from Hamal Lake, Sindh, Pakistan. They were chloroformed in Laboratory for Helminthological examination. The visceral organs were removed and placed separately in petri plates and samples of each organ were examined carefully on dissecting microscope. During study it was observed that all hosts were harbouring large number of specimens of genus *Opisthorchis* Blanchard, 1895 in Liver and Gall Bladder. Every specimen of host was infected with score of genus *Opisthorchis*. Moreover, genus *Opisthorchis* Blanchard, 1895 is reported first time in *Nycticorax nycticorax* from Hamal Sindh, Pakistan.

DISTRIBUTION AND ABUNDANCE OF INDIAN PANGOLIN (*MANIS CRASSICAUDATA*) IN DISTRICT MANSEHRA

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The only pangolin species reported from Pakistan is the Indian pangolin (*Manis crassicaudata*), the armored mammal, that has got localized distribution in the country. The current study aimed at investigating its distribution and population in Mansehra District, where no previous published records of its distribution were available. Distribution of the species was determined by conducting field surveys and recording its direct and indirect signs (such as burrows, fecal matter etc.), and also by getting secondary information from local people using self-designed questionnaires. Population density of Indian pangolin was estimated by counts of its active living burrows at selected sampling sites. Results revealed Indian pangolin occurring at only two sampling sites (site-I; Lassan Nawab and site-VII; Paras) out of total seven selected sites. Very low numbers of feeding and living burrows of the species were recorded at the two positive sites. Only one individual of the species was directly field sighted at site-VIII, Paras. At the two positive sites, Indian pangolin was found more associated with the tree species *Eucalyptus camaldulensis* and *Pinus roxburgii* whereby its burrows were found around these tree species, although *Dodonaea viscosa* was the most abundant shrub species in the habitat of Indian pangolin.

A NEW SPECIES OF GENUS *GANEO* KLEIN, 1905 COLLECTED FROM INDUS VALLEY BULLFROG *HOPLOBATRACHUS TIGERINUS* (ANURA: DICROGLOSSIDAE) OF DISTRICT UMERKOT, SINDH, PAKISTAN.

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A survey was conducted on the helminth parasites of Indus Valley Bullfrog *Hoplobatrachus tigerinus* of district Umerkot, Sindh, Pakistan. A total of 18 hosts were collected from different aquatic habitats and examined for the presence of helminth parasites. During examination of gut contents and visceral organs, three trematodes belonging to genus *Ganeo* Klein, 1905 were recovered from small intestine and identified as *Ganeo kabeeri* n.sp. Present species differs from its congeners on the basis of spinose body slightly tapering anteriorly; subterminal oral sucker larger than ventral sucker; shape of cirrus sac; shape and arrangement of testes; bilobed ovary attached with ventral sucker and posterior testis; distribution and extension of vitellaria; shape and extension of ceca and egg size. On the basis of these differentiating characteristics, a new species *Ganeo kabeeri* is proposed. The name of new species refers to Kabeer Ahmed, the father of first author.

COMPARATIVE STUDIES ON EGG QUALITY CHARACTERISTICS, HEMATOLOGY AND REPRODUCTIVE TRAITS IN RING NECKED AND GREEN PHEASANTS IN CAPTIVITY

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Present study was planned to compare egg quality, hematology and reproductive traits in ring necked and green pheasants in captivity. Eggs of both the pheasant species i.e. *Phasianus colchicus* and *P. versicolor* were collected. Each egg was weighed and its length and breadth was taken. These eggs were divided into three weight groups and were classified as light, medium and heavy weight category. After completion of the incubation, the infertile egg percentage was recorded 48% in ring necked pheasants and 42% in green pheasants. Hatched chick weight increased with increase in egg weight. Increase in wing length varied significantly ($p < 0.05$) in among both the sexes and species from day-old chick to 6-month of age. Similarly, significant ($p < 0.05$) differences in various hematological parameters were also recorded with increase in age.

MORPHOMETRIC OF BATS (ORDER CHIROPTERA) FROM DISTRICT BAGH AND POONCH (RAWALAKOT), AJK, PAKISTAN

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Bats are considered among the least known creatures in Pakistan. The present study is the first attempt to study the bat fauna of the region. The main objective of the study is to find out the diversity of the bats in two districts Bagh and Poonch (Rawalakot) in Azad Jammu and Kashmir. Three consecutive nights were spent in each month in both districts and two districts were sampled in three consecutive months. In addition, mountain caves were checked thoroughly to find out the variety of cave roosting bats. Bat specimens captured through mist netting were identified up to species level on the basis of their morphology, external body and cranial measurements, size and shape of the baculum. The age and sex of captured bats were also determined to find out peak breeding seasons of various bat populations. Bats species i.e. *Pipistrellus effinis*, *Pipistrellus kuhli*, *Pipistrellus dormer*, *Pipistrellus javanicus*, *Pipistrellus fenus*, *Pipistrellus pipistrellus*, *Scotophilis kuhli* and *Scotophilis heathii* were recorded during the present study. The bats of this region of Pakistan have never been recorded and this pioneering study is helpful for bat biologists in future to identify various bat species on the basis of this study. The study is also helpful to redefine current distributional changes and habitat preferences of these bat species. It is also help to compare and verify taxonomy of various bat species captured from the study area.

ASSESSMENT OF AVIAN FAUNA OF DIFFERENT RURAL SITES IN SARGODHA, PAKISTAN

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A comparative study of avian diversity was carried out at the five different rural sites in Sargodha from October 2015 to September 2016. The five sites are Farooka (R1), Dera jara (2), Sajoka (R3), Vijhoka (R4) and UOL (84 NB) (R5) Sargodha. Total 48 birds species with total count of 3349 were observed at all sites. Out of 48 birds species 12 resident, 10 abundant, 3 common visitors, 6 winter visitors, 4 summer breeders and 3 uncommon resident species were observed. The average value of Shannon diversity index for rural site R1 (3.21), R2 (3.37), R3 (3.26), R4 (3.3) and R5 (2.96) was observed. The Evenness of R1 (0.87), R2 (0.92), R3 (0.89), R4 (0.91) and R5 (0.81) was also calculated. The average value of Richness index for R1 (14.16), R2 (12.56), R3 (13.45), R4 (13.35) and R5 (14.83) was also calculated. Similarly the average value of Relative abundance for R1 (10.32), R2 (21.96), R3 (14.32), R4 (15.11) and R5 (7.76) was calculated.

ASSESSMENT OF HUMAN-WILDLIFE CONFLICT IN THE TERRITORY OF RIVER CHENAB, PAKISTAN

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Selected sites in three districts i.e. Sialkot, Gujrat and Gujranwala along river Chenab, Pakistan were surveyed to assess extent of human-wildlife conflict in these areas. The study area was part of tropical thorn forest but a larger portion has been changed into agricultural land or human habitations. Most of the respondents were unaware about the role of wild species in ecosystem, certain respondents disliked wild species in their areas and respondents had positive view about wildlife in the area. The collected data revealed that 42 percent people said that Wild boar was more damaging the crop, while 34% informants said that Asiatic jackal, 11% said that diseases, 6% said that porcupine, 7% said that others including rats, squirrels, crows and sparrows were damaging the crops. The results revealed that 34% informants said that Asiatic jackals are more dangerous than other animals for livestock, while 25% informants said that Indian fox, 10% said that jungle cat and 6% said that raptors birds are more dangerous than other animals.

STUDIES ON GROWTH PERFORMANCE AND FEED PREFERENCES IN MALLARD DUCKS (*ANAS PLATYRYNCHOS*) IN CAPTIVITYNAZIA SULTAN,¹ ARSHAD JAVID,^{1,*} WAQAS ALI,¹ ALI HUSSAIN,¹ SYED MAKHDOOM HUSSAIN² AND MUHAMMAD HAFEEZ-UR-RAHMAN³¹*Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore*²*Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore*³*Department of Zoology, Government College University, Faisalabad*

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An experiment was designed to assess the growth performance and feed preferences in mallard ducks *Anas platyrhynchos* in captivity. Day-old chicks were arranged in to two groups, each containing 10 birds. Each group was considered as a separate treatment and was kept separately into 5×5×4 ft (length× width× height) iron cages, provided with separate feeding and drinking facilities. These cages were placed in a 20 × 20 ft (length× width) well ventilated room. There were two treatment diets i.e. corn (treatment 1 diet) and barley (treatment 2 diet) and were provided to group I and group II birds, respectively. Each bird was tagged individually and its external body measurements viz. body length, beak length, thigh length, wing length and wingspan were recorded at the initiation of experiment and thereafter increase in these parameters was recorded on weekly basis and were compared. The experimental birds were kept in similar climatic conditions like temperature, ventilation, floor space and light for a period of 14 weeks. Significantly higher ($p < 0.05$) beak length, body length and thigh length were recorded in *A. platyrhynchos* fed with treatment 2 (barley) diets while non-significant variations in body weight, wing length and wingspan were observed between *A. platyrhynchos* fed with T1 and T2 diets.

DIVERSITY, DISTRIBUTION AND HABITAT PREFERENCES OF AMPHIBIAN AND REPTILES AT KALABAGH GAME RESERVE, PAKISTAN

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Present survey extending from July, 2015 through March, 2016 was conducted at Kalabagh Game Reserve, district Mianwali, Pakistan. Six amphibian species representing 6 genera and 3 families while 24 reptilian species representing 23 genera and 13 families were recorded from the study area. Hand picking was the most effective method of all the five capture techniques including hand picking, use of snake sticks, forceps, drag nets and noose traps. Shannon-Wiener Diversity Index was calculated as 3.26641 indicating high level of diversity in the study area and Census index was 15 species per km². Cultivated area was highly diverse habitat as compared to other habitat types and 21 species were recorded with most dominant amphibian species was *Bufo stomaticus* with a relative abundance (Pi) of 0.080645 while *Varanus bengalensis* is reptilian dominant species with relative abundance (Pi) of 0.067742. Captured amphibian species included *Bufo stomaticus*, *Microhyla ornata*, *Euphlyctis cyanophlyctis*, *Fejervarya limnocharis*, *Hoplobatrachus tigerinus* and *Sphaeroteca breviceps*. Single turtle species *Lissemys punctata andersoni* was recorded from the study area. Among lizard species *Calotes versicolor*, *Laudakia nupta*, *Trapelus agilis pakistanensis*, *Eublepharis macularius*, *Cyrtopodion montiumsalsorum*, *Hemidactylus barookii*, *Hemidactylus flaviviridis*, *Ablpharus grayanus*, *Eutropis macularia*, *Lygosoma punctate*, *Novoeumeces indothalensis*, *Uromastyx hardwickii* and *Varanus bengalensis* were recorded. Similarly, snake species include *Leptotyphlops macrorhynchus*, *Ramphotyphlops braminus*, *Eryx johnii*, *Amphiesma stolatum*, *Platyceps rhodorachis*, *Lycodon aulicus*, *Ptyas mucosus*, *Bungarus caeruleus*, *Naja oxiana* and *Echis carinatus*. The venomous snake species of the study area include *Naja oxiana*, *Bungarus caeruleus* and *Echis carinatus*.

COMPARISON OF COMPOUND DIET VERSUS SINGLE GRAIN BASED FEEDING RATION ON PRODUCTIVITY OF BUDGERIGARS (*MELOPSITACUS UNDULATES*)

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Eighteen pairs of adult budgerigars (*Melopsitacus undulates*) were randomly divided into three experimental groups having three replicates each and each replicate had two pairs of budgerigars. Ten different types of seeds i.e. millet (*Pennisetum typhoidium*), cheena (*Panicum milaceum*), yellow kangini (*Setaria italica*), red kangini (*Panicum milaceum*), sunflower seeds (*Helianthus annuus*), sunflower seeds (*Carthamus tinctorius*), rice (*Oryza sativa*), charray (*Sorghum propinquum*) available in local market were offered to the budgerigars during acclimatization period of ten days. After this trial two preferred seed diets were chosen for experimental treatment of budgerigars. The group A was treated with millet (*Pennisetum typhoidium*) only which served as

control. Group B was treated with yellow kangini (*Setaria italica*) and group C was treated with combination of millet and yellow kangini in 1:1 ratio. It was observed that birds fed millet and yellow kangini had higher feed intake compared to other treatments. Maximum eggs were laid by the birds fed with millet + kangini (2.37 ± 0.42) and minimum in birds fed with millet (1.70 ± 0.29) and yellow kangini (1.73 ± 0.27), separately. Maximum eggs were hatched by the birds fed with millet + kangini (1.73 ± 0.44) and minimum in birds fed with millet (0.83 ± 0.24) and yellow kangini (0.97 ± 0.26), separately. From the study it can be concluded that budgerigars fed with combination of millet and yellow kangini showed statistically better performance than the bird with the millet or yellow kangini in terms of less feed consumption and high egg production and hatchability ratio.

PUBLIC PERCEPTION ABOUT HERPETOFAUNA AND THEIR EXPLOITATION FOR TRADITIONAL MEDICINE IN DISTRICT KASUR, PUNJAB, PAKISTAN

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Present survey extending from October, 2015 through March, 2016 was planned to document public perceptions towards amphibians and reptiles in district Kasur, Pakistan. A total of 200 persons, 50 from each tehsil were interviewed from all the four tehsils namely Patoki, Chunnian, Kot Radha Kishan and Kasur. The people of the study area used to prepare traditional medicines from body parts of *Bungarus caeruleus*, *Naja naja*, *Ptyas mucosus*, *Eryx johnii*, *Varanus bengalensis* and *Uromastix hardwickii*. More than 80% of the respondents considered amphibians and reptiles as useless animals, 71% as symbol of bad omen, 73% believed that herpetiles are used in traditional medicine and 3% considered that they are also used as food. Different superstitions are also linked with amphibians and reptiles and this mindset lead to unnecessary killing of species. Among all the herpetiles, snakes are most disliked taxa and killed by the locales. Conservation education and awareness campaigns are recommended to avoid unnecessary killing of the amphibians and reptiles of the study area.

RELATIVE EFFECTIVENESS OF DIFFERENT SAMPLING TECHNIQUES USED IN HERPETILES SAMPLING AT DISTRICT KASUR, PUNJAB, PAKISTAN

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The present six month survey was conducted at University of Veterinary and Animal Sciences, Ravi Campus, Pattoki from July through December, 2016 to check the relative effectiveness of three traps i.e pitfall traps, funnel traps and glue trap. A total of 20 pitfall traps, 16 funnel traps and 26 glue traps were installed and monitored in agricultural fields, barren lands and near human habitations. 228 specimens were captured representing 16 species of amphibians and reptiles. Out of these, 73 amphibians specimens belonging 3 species and 3 genera (*Bufo*, *Hoplobatrachus*, *Euphylyctis*) and 2 families (*Bufo* and *Ranidae*) while 154 reptiles specimens representing 13 species (8 snakes and 5 lizards) belonging to 13 genera and 5 families were captured. It has been observed that pitfall trap was most effective trap to capture herpetiles as 57%

amphibian and 58% reptiles were captured through pitfall traps, 22% reptiles with glue traps and 14% reptiles through funnel traps.

**SEASONAL VARIATIONS IN GROWTH PERFORMANCES AND BLOOD
BIOCHEMICAL PROFILE IN RING NECKED PHEASANT (*PHASIANUS COLCHICUS*)
FED WITH DIFFERENT DIETARY REGIMES**

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An experiment was planned to record seasonal variations on growth parameters and blood biochemical profile in ring necked pheasant (*Phasianus colchicus*). Experimental birds were selected at random and were arranged in three groups; each group was considered as a separate treatment and was fed with different diets. Group I birds were fed with poultry feed, group II birds with corn feed and group III birds with millet. The effect of feed and season was assessed on the growth performance and blood biochemical profile of these pheasants. All these birds were kept in cages having dimensions 5 × 5 × 4 feet (length × width × height) provided with separate feeding and drinking facilities and were housed in a well-ventilated 20ft x 20ft (length x width) room. Significant (p<0.05) variations in body weight, body length, body width, beak length and leg length were recorded in *P. colchicus* fed with different treatment diets. However, non-significant variations in tail length, shank length and wingspan were observed between the treatments. Higher body weight, body length, body width and leg length were recorded in ring-necked pheasants fed with treatment I diets. Variations in cholesterol, glucose, total protein, albumin and globulin concentrations were also recorded with reference to age and season. It can be concluded from present study that the growth and blood profile is influenced by the diet and season.

**DIVERSITY AND ABUNDANCE OF VULTURE SPP. AT PIR LASURA NATIONAL
PARK (PLNP), AZAD JAMMU & KASHMIR, PAKISTAN**

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Vultures perform sanitation services in the environment by feeding on carcasses which may otherwise decay and become source of disease spread affecting health of other animals as well as human beings. In the last couple of decades, a drastic decline in the population of vultures was reported, mainly as a result of non-steroidal anti-inflammatory drug diclofenac being used in domestic animals in India, Pakistan and Nepal. The current study aimed at investigating diversity, distribution and abundance of vulture species found in and around Pir Lasura National Park AJ&K. Field surveys were conducted during September 2015 to August 2016, to record diversity and

distribution of vulture species, observations were recorded using binocular, and spotting scope and vulture species were identified using field guide (Birds of Pakistan). The areas having population of vultures were marked as positive and vice versa. The abundance of each vulture species was assessed using colony count method both at dawn and the dusk. Three vulture species were recorded in the study area; Himalayan griffon vulture (*Gyps himalayensis*), Egyptian vulture (*Neophron percnopterus*) and White-rumped vulture (*Gyps bengalensis*). Himalayan Griffon vulture was recorded at eleven different sampling sites, Egyptian vulture at only four sites while the White-rumped vulture also at four different sites. All three species were found distributed from 560 m-1530 m elevation a.s.l. in the study area. Total population of Himalayan Griffon vulture was estimated to be 128 vultures, and its mean colony size ranged between 7-20 individuals. For Egyptian vulture, the total population was estimated to be N= 41 vultures and its colony size ranged between 14-26 vultures. The total population of White-Rumped vulture was estimated to be N = 48 vultures, the mean colony size ranging between 15-23 vultures. The three Vulture species were observed feeding on carcasses of seven different mammalian species and poultry waste. The mammalian carcasses included buffalo (36.66%), cow (23.33%), goat (16.66%), sheep (6.66%), dog (3.33%), jackal (3.33) and mule (3.33%), while carcasses poultry contributed for (6.66%). More detailed studies on ecology of vulture species are required to get better understanding of underline ecological patterns.

**SEASONAL VARIATION IN THE DIET OF THE HIMALAYAN MONAL
(*LOPHOPHORUS IMPEJANUS*) PHEASANT IN MACHIARA NATIONAL
PARK, AZAD JAMMU AND KASHMIR, PAKISTAN**

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Himalayan Monal Pheasant *Lophophorus impejanus* is listed in Appendix-I of CITES. The Machiara National Park (MNP) in Pakistan supports population of this species. Lack of information about its diet is due to distance and inaccessibility to dense forests and mountainous territory. Present study was designed to analyze food composition of Himalayan Monal Pheasant in MNP, Azad Jammu and Kashmir by using microhistological technique. Thirty faecal samples were collected during the summer season and 15 fecal samples during the winter season of 2015. For reference key 41 plant species were collected from the study area. Analysis of fecal samples with the aid of microhistological method showed that *Bergenia ciliata* was the dominant plant throughout the summer period while *Impatiens edgeworthii* was the dominant plant in diet of Himalayan Monal Pheasant during winter season. Besides plants some other materials were also recorded from fecal samples i.e. stones, seed shells, quarts, invertebrate body parts and feathers. The results of this study will be helpful in the management of this species in MNP.

DIET OF HIMALAYAN GRAY LANGUR (*SEMNOPITHECUS AJAX*) IN MACHIARA NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN

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Gray Langur (*Semnopithecus ajax*) belongs to family Cercopithecidae and order Primates. Present study was designed to determine the diet composition of this species in MNP during summer and winter 2015 using microhistological technique. Sixty fecal samples were collected from the study area during summer and winter 2015 (thirty samples during each season). These samples were analyzed for the determination of food composition using microhistological technique. A total of 23 plant species were observed during summer and 15 plant species during winter 2015. During both the seasons *Asculus indica* was found as the dominant plant species in the diet of gray langur having RIV 8.36 and RIV 10.92 in both summer and winter respectively. Diet breadth of all the plant species was also calculated using Levin's measure of niche breadth (B). Diet breadth was higher during the summer season. The present data on diet composition of gray langur will be helpful for conservation of gray langur in Machiara National Park.

IMPACT OF CLIMATIC FACTORS ON HUMAN-COMMON LEOPARD CONFLICT IN GALIAT FOREST DIVISION, DISTRICT ABBOTTABAD

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Human-wildlife conflict is one of the main conservation challenges as it intends to increase persecution of threatened species and also contribute significantly to the loss of range and natural habitat. Any attempts to mitigate human-leopard conflict and to conserve the species should be based on an unequivocal understanding of the conflict patterns. A survey was conducted with an intention to quantify livestock losses resulting from depredation by leopards in the Galiat Forest Division, Abbottabad with respect to climatic factors as climatic factors like temperature, rainfall and elevation play an important role on the pattern of common leopard attacks on livestock and pet animals. Over the past 15 years, total of 2671 livestock and dogs were killed by common leopard in 1186 attacks. The common leopard attacks on livestock were lowest (13.8%) in winter months (November - February) and slightly increased (17.1%) during spring (March - April). Maximum attacks (42.4%) were recorded in summer (May - July) but showed almost 50% decreases (26.6%) in the fall season (August - October). Rainfall was found as one of the important climatic factors influencing human-common leopard conflict in the study area. Rainfall also had direct correlation with the common leopard attacks on livestock. Elevation has also been found as one the key factors affecting human-common leopard conflict. However, there was no relation found between human-common leopard conflict and average elevation in the study area Majority of killed were goats n = 657, followed by sheep n = 424, cow n = 285, buffalo n = 144, donkey n = 220, poultry chicken n = 625 and dogs 316. Most of common leopard attacks 53.8% occurred during the evening time (1700-2100 hours), night time 28.2% (2100-0400 hours), morning time 11% (0400-0900 hours),

and 6.6% occurred at the day time (0900-1700 hours). Approximate distance of attacks was 1-5 km from the reserve forests. Majority of attacks 58.0% occurred on private lands near human settlements followed by attacks in the reserve forests. Total livestock economic losses caused by common leopard over the past 15 years were estimated at Rs. 37.19 million (USD = 354242). In the GFD, total of 32 people became victims of common leopard attacks from 2001 to 2015 and 72 leopards were killed in persecution. Our research suggests that human and leopards can coexist in the area but conflict should be mitigated. For this concerned departments, by only managing leopards and by interacting with local communities, can understand the human-common leopard conflict. Educational outreach can help locals minimize the conflict and to understand their ecological importance. With proper training and mass awareness programs at local level, locals can learn to recognize the behavior of species, and their ecological importance and are the best tools to mitigate human-common leopard conflict.

EFFECTS OF CAPTIVE ENVIRONMENT ON THE FECUNDITY, SURVIVAL AND GROWTH RATE OF GRAY LAKE GOSLINGS (*ANSER ANSER DOMESTICUS*) IN THE KARACHI ZOOLOGICAL GARDEN.

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Captive breeding is a process through which efforts are made to breed in captivity the animals which are endangered or threatened so that they could be introduced in their natural habitats (Curman, 2000). In zoos captive breeding programs are introduced to provide safe and secure environment to endangered animals for mating, reproducing and to increase their population which help in saving the threatened species from extinction and reintroduction of animals back in to the wild areas. Domesticated geese (*Anser anser domesticus*) possess extraordinary abilities to involve into numerous, morphologically and behaviorally breeds within a few generations (Arbuckle, 2005). In the present study, observations were carried in two phases. In first phase, eggs of domesticated geese were counted and the shape, size, hatchability, weight, color and incubation period were observed. In second phase, the goslings were kept in captive environment and the survival, growth rate, feed intakes were observed. Breeding season was started in December and ended in March. Total 93 females were present out of which 72 females laid eggs which were 77.4% of total females. Higher number of eggs laying was observed in February. 22 females incubate their eggs which were 30.55% of the females that laid eggs. Clutch size varied between 5-12 eggs. Hatching of goslings was started in 26th January and ended in 5th April. 55.84% of eggs were hatched while 44.12% were remained un-hatched. Annual survival rate was high (83.72%) while mortality rate was low (16.27%). Measurement of growth and weight was carried out in April upto September i.e from 1st week to 23rd weeks. Initial weight was 100grams which steadily increased in 3rd, 5th and 7th week (33.3%, 50% and 24.32% respectively). Higher growth rate and weight was observed in 5th week (11.6% and 54%). Annual survival was high and illness was observed to be very rare due to the supporting environment of lake. In current study captive environment was found to be very effective reproductive success and hence in conservation of domesticated geese.

**ASSESSMENT OF AVIAN FAUNA OF DIFFERENT RURAL SITES IN
MIANWALI, PAKISTAN**

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A comparative study of avian diversity was carried out at the six different rural sites in Mianwali from October 2015 to September 2016. The six sites are Kamar mushani (R1), Shah allam wala (R2), Gaziwal (R3), Chaq appu khail (R4), Gharuan wala (R5) and Fatima Jinnah girl's school Kamar mushani (R6) Mianwali. Total 127 bird's species with total count of 9585 were observed at all sites. Out of 127 birds species 82 residents, 33 winter visitors, 2 summer visitors and 10 passage migrants were observed. The average value of Shannon diversity index for rural site R1 (2.15), R2 (2.75), R3 (2.42), R4 (2.29), R5 (2.67) and R6 (2.07) was observed. The Evenness of R1 (0.77), R2 (0.84), R3 (0.80), R4 (0.78), R5 (0.81) and R6 (0.80) was also calculated. Similarly the average value of Richness index for R1 (13.83), R2 (26.75), R3 (20.83), R4 (18.83), R5 (26.83) and R6 (13.83) was also calculated.

**POPULATION STATUS, HABITAT AND FEEDING PREFERENCE OF SPOTTED
OWLET (*ATHENE BRAMA*) IN FOOT HILLS OF MARGHALLA HILLS
NATIONAL PARK, ISLAMABAD.**

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The spotted owl (*Athene brama*) a common resident of open habitats including farmland and human habitation, it has adapted to living in cities. The present study were conducted to investigate the population status, feeding and habitat preferences, in Foot Hills of Marghalla Hills National Park, Islamabad. For this tenacity study area divided into Fifteen (15) sampling sites covering six types of habitat that is building with trees, Parklands, forested area, Old trees, earth cutting and agricultural habitat. The data on population, feeding and habitat were collected from August-2015 to June-2016. The maximum number of owl's population density recorded in the months April-June, 2016 which is 1.31/Km² followed by the 1.21/Km² in March, 1.06/Km² in February, 0.88/Km² in January, 0.75/Km² in December, 0.66/Km² in November, 0.61/Km² in October-September and 0.68/Km² in August respectively. Prey frequencies were calculated from pellets; total 149 pellets analysis carried out and found that the major prey item in the food of spotted owl in insects which is 62%, followed by the small mammals which was 45% prey in the pellets. There are 12% prey species birds and 3.30% reptiles and 18% vegetative material and 1.3% polythene bags pieces that indicated high degree of soil pollution in urban environment. The prey abundance of spotted owl which consumed during feeding was House mouse (*Mus musculus*) which was 38% followed by Indian field mouse (*Mus booduga*) 36.24% and House shrew (*Suncus*

murinus) 32.21%. The major prey item in term of prey was Coleoptera (58%), Orthoptera (43%) and Lepidoptera (31%). These two group constitute the major food of spotted owl. The *Bandicoota bengalensis* (2%) which was recorded only on 3 pellets, the *Rattus rattus* (9.39%) in pellets analysis. It was also observed that the spotted owl feed on young ones of *Bandicoota bengalensis* and *Rattus rattus*. There are 6 types of habitat and fifteen (15) selected sites, the population of spotted owl mainly preferred those habitat which have building (crevices use for nesting) with trees (roosting). There are dominant tree species around the habitat of spotted owl were; Blue pine (*Pinus roxburghii*), **Acacia modesta**, **Neem** (*Azadirachta indica*), White mulberry (*Morus alba*), Peepal tree (*Populus alba*), Santha (shrub) (*Dodonaea viscosa*), Kachnaar (*Bauhinia variegata*), Olive tree (*Olea ferruginea*), *Acacia nilotica* and Indian banyan (*Ficus benghalensis*). The higher population density were recorded at Site# S-14 (2.18 Km²), S-01 (1.59 Km²), S-04 (1.40 Km²) respectively. There were observed that during data collection the electric poles, power lines, as well as street lamp poles provide a broad view for the locating prey species in their natural habitat. The pure forested habitat of Marghalla Hills National Park, Islamabad have no owl's population. Those sites that have forested habitat were not preferred by spotted owl like S-03, S-07 and S-13. It was concluded that this species mostly preferred that area residing along the human habitation with gardens or agricultural land that provide a plenty of food and building provide suitable nesting site. Further studies on artificial nest box usage by spotted owl will be carried out for breeding and conservation of this valuable species in the ecosystem.

BIOLOGY OF GREY PARTRIDGE (*FRANCOLIN PONDICERIANNUS*) IN GAME RESERVE OF PIND HASHIM KHAN, DISTRICT HARIPUR, KHYBER PAKHTUNKHWA, PAKISTAN

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The grey partridge (*Francolinus pondicerianus*) was once a common species in World's agricultural landscape but has suffered from massive declines over the last half century. The present study was conducted to measure the current population, threats and its underlying causes, rule of indigenous peoples and application of wildlife rules for conservation of Grey Partridge in Game Reserve of Pind Hashim Khan. The line transect method was used to measured population density. Threats and their causes to Grey Francolin were identified through the comprehensive surveys of questionnaire, interviews and group discussion with the society in the Game Reserve. The result shows that the important cause was the lack of awareness level among the community. But besides it others causes were also identified e.g. overgrazing, poaching, increase in human population, Careless of Wildlife Department for Game Reserve and better production of crops growth. The Density was estimated through March to August in four selected places. The study area was divided into four sub-study sites. The Mean Population Density of Grey Francolin in Transect I (Nartopa), Transect II (NeeliTakki), Transect III (Pharhari) and in Transect IV (Bayian Ahmad Ali Khan) was 0.384/ha, 0.401/ha, 0.389/ha and 0.401/ha, respectively.

**SURVEY OF BIRDS IN WINTER SEASON AT KETI BUNDER,
DISTRICT THATHA, PAKISTAN**

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Keti Bunder is situated Tehsil of District Thatha, Sindh and part of Indus Delta. The objective of study was to know the avifauna diversity in the winter of Keti Bundar. The present study was carried out in winter season. Linear count survey method was used for population estimation of birds from the study area. Both, direct and indirect methods were applied to find out birds diversity of the area. During this survey, total 49 winter season bird's species belong to 33 genera and 21 families were recorded. Total 4280 number of birds was recorded from the Keti Bunder, District Thatha. The statistical analysis for diversity indices were as; Dominance (0.06153), Shannon-Wiener diversity Index (3.237), Simpson Index (0.9385), Margalef Index (5.74) and Evenness (0.5195).

**BIOSYSTEMATIC STUDY OF CHIROPTERA OF DISTRICT SWABI, KHYBER
PAKHTUNKHAWA, PAKISTAN**

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The present study was conducted from April-July 2014 in district Swabi of Mardan Division. Total 8 stations were sampled for bats where total 482 bats were recorded. A total of 5 species of bats belonging to two families, three genera were recorded. These includes the Indian flying fox (*Pteropus giganteus*), the Asiatic greater yellow house bat (*Scotophilus heathii*), the Asiatic lesser yellow house bat (*Scotophilus kuhlii*), the least pipistrelle (*Pipistrellus tenuis*) and the Dormer's bat (*Pipistrellus dormeri*) were recorded throughout the study area. Total 53 individuals were captured. Of these *Pteropus giganteus* (n=18) were captured only from Hemlit Nehir (34°2'57" N 72°38'1" E) and Zaida (34°4'0N 72°28'0E). *Scotophilus heathii* (n=10) were captured from three areas i.e. Tarbela Dam (34° 5' 16"N, 72° 42'2 E), Topi Khawar (34° 4' 17"N, 72° 38'46 E) and Gandaf (34°7'0 N 72°41'0E) while *Scotophilus kuhlii* (n=7) were captured from Batakara (34°2'0N, 72°36'0E), Hemlet Nehir and Maini (34°7'07"N, 72°37'32" E). *Pipistrellus tenuis* (n=8) were captured from Tarbela Dam, Zaida and Hamlet Nehir. *Pipistrellus dormeri* (n=10) were captured from Tarbela Dam, Zaida and Ghulam Ishaq Khan Institute (34° 4' 17"N, 72° 38'46 E).

**STUDY OF AVIAN DIVERSITY IN DISTRICT HATTIAN BALA,
AZAD JAMMU AND KASHMIR (PAKISTAN)**

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A study was conducted from March to November, 2015 to determine the diversity and distribution of avian fauna in District Hattian Bala, Azad Jammu & Kashmir. A total of 50 study surveys was conducted. The area was surveyed in morning and evening when the birds were more active, using fix count method. Data were recorded by using binocular and digital camera and identified with the help of field guides. The results showed that the species belonging to order Passeriformes (59.53%) were more abundant followed by Piciformes (7.40%), (Charadriiformes (7.40%), Columbiformes, (3.70%) Cuculiformes (3.70%), Psittaciformes (3.70%), Accipitiformes (3.70%), Apodiformes (3.70%), Bucerotiformes (3.70%), (3.70%), and Strigiformes (3.70%). The most common birds recorded in the study area and its surroundings were House sparrow followed by Common myna, White browed bulbul, White bared bulbul, Jungle crow, House crow, Himalayan bulbul and etc. Diversity indices were recorded highest in summer (1.36) and lowest in autumn (1.24). Overall relative abundance was recorded highest for House sparrow (1.153) and lowest for River tern (0.0005). Species richness was recorded highest in summer (n=41) and lowest in autumn (n=38). The children and women, during wood cutting destroy the nests and eggs of birds that cause decline in bird species. Government and non-government agencies should launch an awareness programs for local people to highlight the importance of bird species in ecosystem that will result in better conservation of local avian fauna.

**RECENT RECORD OF GREATER FALSE VAMPIRE BAT, *MEGADERMA
LYRA* FROM PUNJAB, PAKISTAN**

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The greater false Vampire bat *Megaderma lyra* is found in many different biotypes. The *M. lyra* is an Indomalayan bat species. It has been reported first time from the plateau region of northern Punjab where 13 greater false vampire bats were roosting in dark doomed shaped room of fort Rohtas of district Jehlum. We captured the six specimens from the roosting site by using the hand net. During the observation it was found that all the captured specimens were male. These bats have unique facial features, an erect and elongated nose-leaf, large oval ears that joined on the forehead and without tail. We have taken the morphometric measurements of all the captured specimens. External, cranial, and bacular measurements of *M.lyra* are compared with the previous available literature. The average head and body length of all 6 specimens was 80 (78-83) mm, forearm was 67 (65-69) mm long, lengths of 3rd, 4th and 5th metacarpals 51.7 (51-53), 55.1(54-

56.7) and 60.4(58.7-62.4) mm were respectively. The greatest skull length (n= 2) 29.8 (29.3-30.4) was mm, breadth of braincase was 12.7 (12.4-13) mm while bacular length of a male specimen was 3.075mm.

DIET PREFERENCES AND GENERAL BEHAVIOR OF PEAFOWLS IN CAPTIVE ENVIRONMENT

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Animals kept in captivity tend to behave differently due to confinement. It is the responsibility of the animal keepers to provide for proper space and food to the animals for their welfare. UVAS, Ravi campus houses eighteen peafowls in different enclosures and include Indian peafowls, Java green peafowl, and white peafowl and also cross breeds of Java and Indian peafowls. The present study was conducted to observe the diet preferences and general behaviour of these peafowls under captive conditions. Three different diets based upon the availability of food items were selected and fed to the birds over a duration of one week each. During the fourth week, all three diets were combined and offered to the peafowls. The feed was pre-weighed and the left-over feed was collected and weighed the next day before offering new feed. The diet preference was noted to be in the order of Maize > Millet > Poultry feed which contradicted the verbal reports of animal keepers that poultry feed was consumed in larger amounts. During feeding the behavioral patterns of the captive peafowls were monitored on daily basis. Time spent in different behavioral activities was noted in seconds using the stopwatch. On average most time spent by all species and varieties of peafowls was in walking around within the enclosure (20.6%), followed by litter pecking (13.8%), feather pecking (13.0%) and standing (12.7%). Feeding and drinking consumed 4.6% and 2.1% of time budget respectively.

STABILIZATION AND DEGRADATION OF SOIL OWING TO ITS PH AND MACRO- FAUNA DYNAMICS IN TOMATO FIELDS

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The present study was conducted to examine the role of soil pH in stabilization and degradation of soil macro-fauna dynamics in tomato fields. After completion of tiresome trails of data collection to data analysis as prescribed in methodology, the recorded taxa composition as follow: a total of 1086 specimens belonging to 95 species and 12 orders were collected from the both fields of tomato (treated and control) during the whole crop season for research, comprises on seven sampling trails. A large number of specimens were founded from tomato control field with relative abundance of 64.27% (N = 698) and 35.73% (N = 388) were recorded from tomato treated field with relatively lesser number of individuals. From fields of tomato control a total of 64 species belonging to 59 genera from 33 families and 11 orders all over dominated over the collections from treated fields of tomato crop that consisting of 11 orders, 33 families, 49 species belonging to 45 genera of all above

taxonomical groups. In tomato control fields, relative abundance of *Callobius pictus* (Aracnidae: Amaurobiidae) 27.79% (N = 194) was quite high in according to other recorded taxa. From tomato treated fields, maximum relative abundance 44.33% (N = 172) was recorded for *Diachlorus ferrugatus* (Diptera: Tabanidae). From tomato treated fields, maximum relative abundance was recorded for genus *Diachlorus* (Family: Tabanidae) 44.33% (N = 172). Whereas, from tomato control fields, highest relative abundance 27.79% (N = 194) was recorded for genus *Callobius* (Family: Amaurobiidae). From total of 50 families; 33 families were recorded for tomato control field. Maximum relative abundance was presented for family Amaurobiidae (Order: Aracnidae) 27.79% (N = 194). In tomato treated fields a total of fifty families were recorded among which family Tabanidae (Order: Diptera) was establishing maximum relative abundance 44.33% (N = 172). The results of ANOVA between depending variables (Tomato Control and Tomato Treated) and independent variable (Soil macro-fauna) were non-significant (F = 1.10; P = 0.3049).

EFFECT OF SEASON AND AGE ON INDIAN RED JUNGLE FOWL (*GALLUS GALLUS MURGHI*) SEMEN CHARACTERISTICS: A 4-YEAR RETROSPECTIVE STUDY

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The reproductive potential of the cocks is speculated to vary with age/season and largely differ not only in closely related species but even in subspecies, breeds and/or strains of the same species. The knowledge about retrospective seminal parameters is therefore the prerequisite to achieve maximum production potential of at-risk species for *ex situ in vitro* conservation. A 4-year study was designed to evaluate the effect of age and season on semen characteristics of Indian red jungle fowl (*Gallus gallus murghi*) in retrospective manner. Semen ejaculates (n=1148) were regularly collected (spring, summer, autumn, winter) from eight adult Indian red jungle fowl observed from their first to their fourth year of age (6-54 months). Different semen quantitative and quality parameters viz; volume (μ l), concentration (1×10^9 mL⁻¹), total sperm per ejaculate (1×10^9 mL⁻¹), motility (%), livability (%), plasma membrane, acrosome integrity (%) and semen quality factor were routinely evaluated. Results described a chronological increasing trend with age for most sperm quantitative and qualitative traits (semen volume, sperm concentration, total sperm per ejaculate, plasma membrane integrity, livability, acrosomal integrity, semen quality factor). The highest values were observed at four years of age (P < 0.05) with the exception of sperm motility that was not affected by the age. Spring was the best season for many sperm parameters (volume, motility, plasma membrane integrity, livability and viability; P < 0.05) but a remarkable sperm production was noticed over the year. It is concluded that Indian red jungle fowl exhibit an evolution of sperm production that greatly differs in many points from other subspecies of red jungle fowl and their descendents. It is suggested that semen ejaculates of maximal quality be achieved at age of four years in the spring season for semen banking.

**EXISTENCE OF SOIL MACRO-FAUNA AMONG TOMATO
(*SOLANUM LYCOPERSICUM* L.) AND CAULIFLOWER
(*BRASSICA OLERACEA* L. VAR *BOTRYTIS*) FIELDS**

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During the present study, taxa composition was recorded as follow: among both crops, total 1026 specimens were collected during entire sampling (07 sampling from each category) and maximum population was recorded from tomato fields 53.99% (N=554) and least from cauliflower fields 46.01% (N=472). Among Annelida, Pulmonata (29.06%) and Haplotaxida (22.92%), while among Arthropoda, Coleoptera (27.79%), Isopoda (10.64%), Dermaptera (3.79%), Araneae (2.35%), Diptera (1.63%), and Hymenoptera (1.08%). Whereas in the tomato contain two phyla, 11 orders, thirty seven families and sixty five species. Among Annelida, Pulmonata (29.06%) and Haplotaxida (22.92%), while among Arthropoda, Coleoptera (27.79%), Isopoda (10.64%), Dermaptera (3.79%), Araneae (2.35%), Diptera (1.63%), Hemiptera and Hymenoptera (1.08%). Wherein the population means per sampling was also calculated along with standard deviation (SD). In case of cauliflower fields, maximum population was recorded during 3rd sampling (163±7.799), followed by 156±7.62 (2nd Sampling), 75 ± 4.51784 (6th sampling) and so on. While, least values were recorded during 1st, 4th, 5th and 7th sampling (69 ± 53.32). During the first sampling, temperature was 31°C and humidity 34.5%; then in next sampling temperature was 43 °C and humidity 18%, and 37°C, and humidity 27.5% and so on. In case of tomato fields, maximum population was recorded during 3rd sampling (126 ±3.478), followed by 98 ±4.70638 (6th sampling), 72 ±2.77333 (5th sampling), 57±2.96072 and 7th sampling so on. While, least value was recorded during 2nd sampling (34± 1.25135). Diversity (H') was recorded maximum among tomato (3.665) and least was recorded among cauliflower (2.934). Evenness ratio was also recorded in same context (0.1143 and 0.2222, respectively). Evenness ratio was also recorded in same context from tomato (0.601) and from cauliflower (0.553). Dominance was recorded maximum from cauliflower (0.069) and least from tomato fields (0.045) *t*- value was recorded as 10.91 and degree of freedom was 889.8. Analysis of Variance (ANOVA) was among both fields (cauliflower and tomato) showed non-significant results (F = 0.819; P = 0.371).

AVIAN DIVERSITY OF CAMPUS UNIVERSITY OF PESHAWAR

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University of Peshawar (area of campus about 4 km²) is located in West of Peshawar between 34°00'05.30" N and 71°29'13.69" E. The campus area has diverse habitats including botanical gardens, residential areas, market areas, agricultural field lands, academic blocks and a canal passing through it, providing a diversity of habitats for birds' fauna at a single place with water channel, vegetation and food. A preliminary survey of the avian fauna of university of Peshawar campus was done in 1993. A total of 23 species of birds were reported. The present study

was done to know about the avian fauna at present times. A survey was made from June to October 2016. Data was collected by point method. Avian fauna was observed at weekly basis from 5:30 am to 12:00 noon. Observations done by using binoculars 8 x 40 and by taking pictures from a semi-professional camera. Frequency of birds' sightings was made by counting the number and adding the results at the end. To attract the birds for close observations, food items like pulses, grains and bread crumbs were spread on sites. In present study a total of 34 species belonging to 11 orders and 24 families were recorded. Out of 34 reported species, 13 species were added to the previous records whereas two species previously reported were missing this time. The new addition of species are *Sturnus vulgaris*, *Sturnus contra*, *Acridotheres ginginianus*, *Lonchura punctulata*, *Copsychus saularis*, *Bubulcus ibis*, *Egretta garzetta*, *Ardeola grayii*, *Jynx torquilla*, *Dinopium benghalense*, *Merops philippinus*, *Vanellus indicus* and *Actitis hypoleucos*. The missing species are *Psittacula eupatria* and *Dendrocitta vagabunda*.

SOIL SALINITY AND RELATED SOIL MACRO-FAUNA AMONG CAULIFLOWER (*BRASSICA OLERACEA* VAR. *BOTRYTIS*) FIELDS

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The present research work was conducted to accord "Soil salinity and related soil macro-fauna among Cauliflower (*Brassica oleracea* var. *botrytis*) fields". Among both fields, total 901 specimens were collected during entire sampling (7 sampling from each category) and maximum population was recorded from cauliflower control fields 67.92% (N=612) and least population was recorded from cauliflower treated fields i.e. 32.08% (N=289). In case of cauliflower control fields, maximum population was recorded during 2nd sampling (215±90.21), followed by 145±40.71 (3rd sampling), 86±1.01 (4th sampling) and so on. While, least values were recorded during 1st, 6th and 7th sampling (48±27.88), (32±39.19) and (24±44.85), respectively. Whereas, species abundance was recorded greatest during 2nd and 3rd sampling (19 and 16 species, respectively) at temperature and humidity of 43°C and 18% and 37°C and 27.5%, respectively. However, least species relative abundance was recorded during 7th sampling i.e. 04 species at 38°C (temperature) and 43% (humidity). In case of cauliflower fields, maximum population was recorded during 6th sampling (134±53.34), followed by 99±28.59 (2nd sampling), 54±3.23 (3rd sampling), 44±10.30 and so on. While, least value was recorded during 1st sampling (2±40.00); whereas species abundance was recorded utmost in 4th sampling (12 species) and was recorded equal in 2nd and 3rd sampling (11 species) at temperature and humidity 30°C, 34.5%, 43°C, 18% and 37°C, 27.5%, respectively. However, none of the species was recorded in 1st sampling i.e. 0 species at 31°C temperature and 34.5% humidity. Dominance was recorded maximum from cauliflower control fields (1.0549) and least from cauliflower treated fields (1.0294). However, richness was a little bit recorded high among cauliflower treated fields (11.6633) and least among cauliflower control fields (10.0333). Kruskal-Wallis test was again non-significant (F = 0.43; P = 0.5167). Wilcoxon Rank Sum test was showed that macro fauna were not differ significantly between both fields but habitat preference was level of significance (P-value = 0.5205).

SOIL MACRO- INVERTEBRATES AMONG CAULIFLOWER (*BRASSICA OLERACEA* VAR. *BOTRYTIS*) AND TOMATO (*SOLANUM LYCOPERSICUM* L.) FIELDS

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During present research experiments, among cauliflower total 52 species were recorded belonging to 10 orders 29 families and 41 genera. Whereas among tomato total 53 species were counted pertaining to 11 orders 33 families and 48 genera. Among both fields total 565 specimens were collected during entire sampling (7 sampling from each category) and maximum population was recorded from cauliflower field 47.43% (N = 268) and least population was recorded from tomato i.e. 52.56% (N = 297). In case of cauliflower field maximum population was recorded during 4th sampling (39.39) ± 94, followed by (24.95) ± 3 (1st sampling), (19.59) ± 66 (6th sampling) and so on. While, least and equal value was recorded during 5th sampling (3.74) ± 33. Whereas species abundance was recorded in 6th sampling (17 species) at temperature and humidity 39°C and 41.5 respectively. However, least species abundance was recorded during 1st sampling i.e. 3 species at 31°C temperature and 34.5% humidity. In case of Tomato fields, maximum population was recorded during 6th sampling (11.72±59.00), followed by 71.11±143.00 (7th sampling), 3.94±48.00 (2nd sampling), 14.45±22.00 (3rd sampling) and 22.22±11.00 (1st sampling). While, least value was recorded during 4th and 5th sampling (25.05±7.00) whereas species abundance was recorded utmost in 6th and 7th sampling (61 and 28 species, respectively) at temperature and humidity 38 °C, 78% and 41 °C, 73% respectively. However, least species abundance was recorded during 4th and 5th sampling i.e. 4 species at 35°C; 61% humidity and 37°C and 42% humidity. From the overall findings, significant results were recorded in case of order Hymenoptera from cauliflower fields and in case of Haplotaxida from tomato fields over the entire study period. The relative abundance was recorded maximum from cauliflower fields for order Hymenoptera (41.79%) and least for order Dermaptera and Lithobiomorpha (0.37%). Whereas, in case of tomato relative abundance was recorded maximum from tomato fields for order Haplotaxida (21.21%) and least for order Lithobiomorpha and Orthoptera (1.01%). Moreover, from the entire observations that population of order Hymenoptera was high among cauliflower and in case of tomato field population of Haplotaxida was high. Analysis of Variance (ANOVA) was among both fields (Tomato and Cauliflower) showed non-significant results (F = 0.05; P = 0.8336).

A STUDY OF BIRD DIVERSITY IN DIFFERENT HABITATS OF BALAKOT CITY, KHYBER PAKHTUNKHAWA (PAKISTAN)

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We conducted study surveys (n=65) from March to November, 2015 for distribution and diversity of bird species in different habitats of Tranna, Basoot, Balakot, District Meansehra, Khyber Pakhtoonkhwa (Pakistan). We recorded and identified total 56 bird species, belonging to 14 orders and 36 families. The birds were watched using binocular, and for identification at species

level, we followed Woodcock (1980) and Ali and Riplay (1987). The bird species recorded during the study belonged to the order Passeriformes, Columbiformes, Coculiformes, Accipitriformes, Apodiformes, Psittaciformes, Cathartiformes, Charadriiformes, Ciconiiformes, Coraciiformes, Gruiformes, Pelecaniformes, Strigiformes and Suliformes. The most common birds recorded at Balakot and its surroundings were House Sparrow, White Cheeked Bulbul, Red Vented Bulbul, Common Myna, Crow, and many other species. Species richness was recorded highest in spring and lowest in autumn. Overall relative abundance of these species was estimated between 0.0004 - 0.225. Seasonal diversity indices of the species were highest in autumn (1.1244), followed by summer (1.054), and spring (1.016). We recorded highest diversity indices in forest land (1.145), followed by agricultural land (0.9913) and riverine tract (0.9909). People of the area have indifferent attitude about the importance of bird species. There is a need to involve concerned quarter for taking conservation measures for bird species in the area.

SOIL ACIDITY AND OCCURRENCE OF SOIL MACRO-FAUNA AMONG CAULIFLOWER (*BRASSICA OLERACEA* L. VAR. *BOTRYTIS*) FIELDS

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The present research work was conducted to accord "Diversity and relative abundance of soil macro-fauna on cauliflower treated (*Brassica oleracea* L. var. *botrytis*) and cauliflower control (*Brassica oleracea* L. var. *botrytis*) under the ecological conditions of district Faisalabad (Punjab), Pakistan among acidic soil. Among cauliflower control fields, total 36 species were counted pertaining to 09 orders, 25 families and 36 genera: whereas, among cauliflower treated fields, total 48 species were recorded belonging to 10 orders, 27 families and 44 genera. Among both fields, total 947 specimens were collected during entire sampling (7 sampling from each category) and maximum population was recorded from cauliflower control fields 66.35% (621) and least population was recorded from cauliflower treated fields i.e. 33.65% (315). Wherein in case of cauliflower control fields, maximum population was recorded during 2nd sampling (188 ± 70.21), followed by 121 ± 22.83 (3rd sampling), 102 ± 9.40 (1st sampling) and so on. While, least and equal values were recorded during 4th, 5th, 6th and 7th sampling (11 ± 54.95). However, species abundance was recorded utmost and equal during 2nd and 3rd sampling (20 species) at temperature and humidity 43°C & 37°C and 18% & 27.5% respectively. Nevertheless, least species abundance was recorded during 7th sampling i.e. 4 species at 38°C (temperature) and 43% (humidity). In case of cauliflower treated fields, maximum population was recorded during 4th sampling (94 ± 34.65), followed by 67 ± 15.56 (6th sampling), 38 ± 4.95 (5th sampling) and so on. While, least and equal values were recorded during 1st, 2nd, 3rd and 7th sampling (2 ± 30.41). Whereas, species abundance was recorded utmost in 4th and 6th sampling (27 species) at temperature and humidity 30°C, 39°C and 34.5%, 41.5%, respectively. However, least species abundance was recorded during 1st sampling i.e. 01 species at 31°C temperature and 34.5% humidity. However, from the overall findings, significant results were recorded in case of order Lepidoptera. Diversity was recorded maximum among cauliflower control fields (1.0566) and least was recorded among cauliflower treated fields (1.0315). Diversity_{Maximum} was also higher among cauliflower control fields (2.8021) and least was recorded again in cauliflower treated fields (2.4983). However, richness was again recorded high among cauliflower control fields (14.2604) and least among cauliflower treated fields (10.8734).

AGRICULTURAL PONDS PROVIDING CONTAMINATED HABITATS TO AMPHIBIANS IN DISTRICT LARKANA, SINDH-PAKISTAN

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Environmental contamination is one of the most dreadful factors which lead amphibians to massive decline; therefore an analytical study was carried out in District Larkana to confirm value of some main physico-chemical parameters into aquatic habitats where amphibians spawn and develop. Among other physico-chemical parameters the value of electric conductivity (EC) and total dissolved solids (TDS) was measured using conductivity meter (Model: Orion. 115). Concentration of total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl) and carbon dioxide (CO₂) was recorded through specific titration methodology, whereas quantity of sulphate (SO₄), phosphate (PO₄), nitrite (NO₂) and nitrate (NO₃) was analyzed via ultra-violet (visible) spectrophotometer (Model: Hitachi 200). This study revealed contaminated status of entire 26 amphibian habitations throughout the study period. The value of parameters was noted as followed: EC uS/cm (1180.5-7472.6), TDS mg/L (788.8-4309.2), T-Hard mg/L (200.5-980.7), T-Alk mg/L (150.2-477.5), Cl mg/L (175.3-745.1), SO₄ mg/L (200.8-817.9), PO₄ mg/L (200.0-780.8), NO₂ mg/L (0.9-19.5), NO₃ mg/L (1.3-20.6) and CO₂ mg/L (10.0-26.0). Seasonal variation in values of all parameters was parallel except CO₂ as maximum and minimum values of other parameters was recorded respectively in July and October but utmost value of carbon dioxide was noted in October and bottommost of it was recorded in July. This deplorable condition of study area may have negative impact on the amphibian fauna if not treated urgently.

AQUATIC AND TERRESTRIAL BIRDS OF NAROWAL

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A study on the avian diversity of Narowal was conducted. The area around River Ravi near Narowal was surveyed from September to December, 2016 and Point count method was employed to determine the avian species. The total number of species observed during the surveys was 42 among whom 3 were summer breeders, 2 were passage migrants, 15 were winter migrants while 22 were year round resident species. The Shannon Weiner Index value of 3.17 and Simpson's Index value of 0.94 indicated a high level of diversity to occur in the area. The most abundant species recorded from the area were Common Myna (*Acridotheres tristis*, 14.8%), House Crow (*Corvus splendens*, 8.8%), Cattle Egret (*Bubulcus ibis*, 8.08%), Red Wattled Lapwing (*Vanellus indicus*, 7.8%) and Great Egret (*Casmerodius albus*, 5.5 %). Although the diversity was indicated to be high, most of the species were generalist feeders and commonly found in many areas.

**EMPORAL SERRATIONS OF SOIL MICRO-SITES EVALUATION
OF SOIL MACRO-FAUNA IN TOMATO FIELDS**

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The present study was conducted to record “Temporal serrations and micro-site evaluation of soil macro fauna in tomato fields” under the ecological conditions of district Faisalabad (Punjab), Pakistan. From boundary of the field, total 51 species were recorded belonging to 13 orders, 29 families and 42 genera; whereas from middle of these two sites, total 55 species were counted pertaining to 12 orders, 32 families and 46 genera; while from center of the field, total 36 species were counted belonging to 13 Orders, 22 families and 29 genera. Among three areas, total 1432 specimens were collected during entire sampling (07 sampling from each category) and maximum population was recorded from boundary of the field 45.11% (N = 646), followed by center area 28.77 % (N = 412), and least population was recorded from middle these two sites i.e. 26.12% (N = 374). In case of boundary of the field, maximum population was recorded during 7th sampling (211 ± 83.94), followed by 16 ± 49.29 (6th sampling), 97 ± 3.33 (2th sampling) and so on; while, least values were recorded during 1st sampling (20 ± 51.12). Whereas, species abundance was recorded during 6th sampling (19 species) at temperature and humidity of 17°C, 80% - however, least species abundance was recorded during 1st sampling i.e. 04 species at 13°C (temperature) and 50% (humidity). In case of middle of both sites, maximum population was recorded during 5th sampling (124 ± 49.90), followed by 78 ± 17.37 (4th sampling), 66 ± 8.89 (3th sampling) and so on. While, least value was recorded during 2nd sampling (10 ± 30.71); whereas species abundance was recorded maximum in 5th sampling (16 species) at temperature and humidity 7°C, 100%. However, least species abundance was recorded during 6st and 7th sampling i.e. 05 species at temperature and humidity 17°C, 80% and 22°C, 72%, respectively. In case of center of the field, maximum population was recorded during 5th sampling (127 ± 48.18), followed by 82 ± 16.36 (6th sampling), 63 ± 2.93 (4th sampling) and so on. While, least value was recorded during 2nd sampling (3 ± 39.50) – whereas species abundance was recorded in 5th sampling (11 species) at temperature and humidity 7°C, 100%. However, least species abundance was recorded during 2st sampling i.e. 2 species at temperature and humidity 18°C, 60%, respectively. From total of 43 recorded families, 29 were recorded from boundary and among them, extra ordinary relative abundance was recorded for Succineidae family (54.95%; N = 355) and then maximum relative abundance was recorded for Formicidae family (12.38%; N=80), followed by Porcellionidae (7.28%: N = 47), Lycosidae (5.73%; N = 37), Subulinidae (3.25%; N = 21), Tenebrionidae (2.48%; N = 16), Forficulidae (2.01%; N = 13), Cimicidae (1.70%; N = 11), Carabidae (0.15; N = 10). From total of 43 recorded families, 32 were recorded from middle area and among them, relatively higher abundance (35.56%; N = 133) was recorded for Succineidae family. Thereafter, relative abundance was recorded for Formicidae (18.45%; N = 69), Trichoniscidae (9.36%; N = 35), Lumbricidae (4.28%; N = 16), Trachelipodidae (4.01%; N = 15), Gryllidae, Cylisticidae (2.00%; N = 62), Mymaridae (1.58%; N = 49), Coccinellidae (1.19%; N = 37), Cicadellidae, Scarabaeidae (3.21%; N = 12). From total of 43 recorded families, 21 were recorded from center area and among them, relatively higher abundance (43.93%; N = 181) was recorded for Formicidae family. Thereafter, relative abundance was recorded for Succineidae (26.46%; N = 109), Lycosidae (10.68%; N = 44), Coccinellidae, Trachelipodidae (3.40%; N = 14). Diversity (H') was recorded maximum from middle area (1.7978), followed by center area (1.5174) and least was recorded from boundary area

(1.4559). Dominance was recorded maximum in boundary area (0.4819), followed by again center area (0.4197) and least (0.3013). However, richness was a little bit recorded high among middle area (15.5811), followed by boundary area (14.8487) and least in center (11.3230). Analysis of Variance (ANOVA) was among among tomato fields (boundary, middle and center) showed non-significant results ($F = 0.32$; $P = 0.7297$).

BIOLOGY OF COASTAL VILLAGE OF KARACHI ALONG WITH CHECK LIST OF COMMON BIOLOGICAL LIFE

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The author is exploring marine life for economic utilization since 30 years. In this connection he use to visit different coastal villages. During some of the visits the author remains successful and sometimes become unsuccessful. The author is having different task for visiting coastal village. Some time he is in the dire need of sandy cost or rocky coast or muddy coast, etc. When he need muddy fauna flora and he found that the newly visited beach is muddy. Hence, he fails to collect muddy fauna i.e. tube anymoon, sand any moon, fan worm, muddy skipper fish, etc. All in all, such unsuccessful stories are become very common in the life of author, while visiting new coastal areas. Therefore, the author feels serious pain that this should not happen with the other researcher and entrepreneurs. In this connection the author compiled a check list of the biological life of common Karachi Coast. The Karachi coasts are largely found sandy, muddy, and rocky. Some of the coast are having two qualities i.e. sandy and rocky like KAKA PIR Village of Hawxbay. In this study the seasonal fauna and flora are also recorded in the tables.

MORPHOMETRICS, HABITAT AND BREEDING OF SOUTH-EAST ASIAN TOAD (*DUTTAPHRYNUS MELANOSTICTUS*) IN RAWALPINDI-ISLAMABAD, PAKISTAN

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We conducted the present study to examine morphometric measurements, habitat and some aspects of breeding of data deficient anuran species- South-east Asian Toad (*Duttaphrynus melanostictus*) in Rawalpindi-Islamabad area. The morphometric measurements of the toad showed that the males were 31.12-63.67 mm in snout vent length (49.12 ± 3.47 , $n = 11$) while the females were 38.67-98.12 mm (57.93 ± 5.51 , $n = 9$). Of the 14 measurements tested for male and female toads, the factor analysis produced six (head width, forelimb length, femur length, width of upper eyelid, hand length and distance between nostrils) significant measurements ($r > 0.80$) for male while eight (head length, distance between nostrils, hand length, femur length, tibia length, tarsus and foot length and foot length, inter-nasal space) for female. Based on our data, we characterized the habitat of the toad as having muddy to stony substrate; water with neutral to slightly alkaline pH, less total dissolved solids, low electric conductivity and less turbid; dominated by herbs: *Cannabis sativa*, *Amaranthus* Spp., *Parthenium hysterophorus*; shrubs: *Dodonaea viscosa*, *Lantana camara*; grasses: *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Dichanthium*

annulatum and hydrophytes *Alternanthera sessilis*, *Centella asiatica* and *Polygonum* spp. The breeding of the toad did not confine to monsoon season. The mean air and water temperature during the breeding period were 27°C-30.5 °C and 25°C-28.5 °C, respectively. The toad laid eggs in small sized pools with lentic to slow-moving water with mean depth of 32-64 cm. The males were more abundant than females (2.14♂:1♀) at the breeding sites.

**EFFECTS OF CLIMATE CHANGE ON DIVERSITY, DISTRIBUTION,
HABITAT UTILIZATION AND BREEDING OF BIRDS AT
SIR BANI YAS ISLAND, ABU DHABI, UAE**

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Birds are recognized as one of the most important indicators of the state of the environment. Because they are sensitive to habitat change and they are easy to census, changes in bird populations are often the first indication of environmental problems. Sir Bani Yas Island in the UAE (8700 ha; IBA criteria 2i & 4) is the important bird area and a wildlife reserve. There is now ample evidence of the ecological impacts of recent climate change, from polar terrestrial to tropical marine environments, the responses of both flora and fauna span an array of ecosystems and organizational hierarchies, from the species to the community levels. Phenological responses to climate change differ across trophic levels, which may lead to birds failing to breed at the time of maximal food abundance. Specific groups of birds are at high risk from climate change: migratory, mountain, island, wetland, Arctic, Antarctic and seabirds. The current study is designed to monitor the effect of various climatic conditions and changes affecting bird ecology, including diversity, habitat utilization, migratory patterns and time budgeting. Birds were observed twice a month basis while walking or driving on transects at very low speed and data was recorded on a data sheet for each recording. Especially for water birds, vantage points were selected for observation and recording. From a vantage point for a fixed period of time birds were counted. 8 transects were used following Coastal Areas and mangroves, Pastures and open plains, Mountains and Forest each habitat included 2 transects of 200 m wide and 800 m long. A total of 100 bird species were recorded belonging to 14 orders and 38 families. Moreover, the effects of infrastructure development on the above mentioned parameters were studied. The data was collected through visual observations through binoculars and spotting scopes. Birds were identified through standard field guides. The data on climatic factors were collected through a weather station at Sir Bani Yas Island. Preliminary studies show a significant decrease in the overall population, abundance and diversity of the avifauna at Sir Bani Yas Island.

IODIVERSITY DETERIORATION IN DAMAN VALLEY DUE TO CLIMATE CHANGESALMA SHAHEEN¹, MUHAMMAD MANSOOR² AND MUHAMMAD AHMAD³¹*Department of Soil & Environmental Sciences, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan*²*Arid Zone Research Institute, PARC, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan*³*Agricultural Training Institute, Karor Lal Eason, Layyah, Punjab, Pakistan*

Daman valley is comprised of Dera Ismail Khan, Tank and South Waziristan Agency and its periphery areas. It is chiefly characterized by its sand dunes, wind cyclones, abundant sun shine and common drought prevalence. Under ground water is usually brackish, unsuitable for drinking and irrigation purpose. There is rampant occurrence of torrential, unpredictable and erratic rainfalls in monsoon leaving no chance of infiltration of water for storage. Temperature fluctuation of day and night adversely affected the pollination process in plants. Scarce pollination mean low fruits. Low fruit mean low yield. Honey production has also decreased. Long drought spells due to climate change extended the chances of diseases and insect pest infestation in plants. Long drought spells due to climate change have extended the chances of citrus canker in Citrus plants and malformation in mango. It has also been observed that infestation of yellow rust (*Puccinia striiformis* L.) in wheat and barley due to high humidity has increased through many folds as compared to previous years in this territory. Correspondingly, temperature fluctuation of day and night have tremendously increased infestation of wilt and blight in chickpea. It has been further observed that even the common species of Jand (*Prosopis cineraria* L.), Khaggal (*Tamarix aphylla* L.) and Puth Kanda (*Acyranthus aspera* L.) were diminishing gradually from this sector. Similarly, rangeland flora like Shisham (*Dalbergia sissoo* L.), wild cucurbit (*Mukia maderasputana* L.) and Puncture Clover (*Tribulus terrestris* L.) are not seen in the nomadic life of Daman valley. Deforestation in Daman valley have diminished variety of reptiles, amphibians, birds and mammals that used to feed and live in the habitat of these trees. There is need to launch a comprehensive campaign, on war footing basis, for awareness among public under the one umbrella integrating all the departments of agri-extension, wildlife, agricultural engineering, plant protection, food and nutrition and forestry from provincial set up to mitigate the adverse effects of climate change of Daman valley. It is also suggested that different types of wildlife parks in different locations of Daman valley may be established, where all diminishing plant and animal species may be reared under controlled conditions so that our next generations may see them in alive form.

**A NOTE ON MORPHOMETRIC MEASUREMENTS OF BURROWING FROG
(*SPHAEROTHECA BREVICEPS*) FROM NORTH PUNJAB**

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The Burrowing Frog (*Sphaerotheca breviceps*) inhabits a variety of habitats, including grassland, scrublands, areas of human habitation and temporary wetlands in the breeding season. The present study was designed to study morphometric measurements of Burrowing Frog in areas of North Punjab. The specimens were collected using dip net and seldom captured with hands. The

specimens were later anaesthetized using chloroform, fixed and preserved in 10% formalin. The min.-max. range (mean \pm S.E.) of body length (mm) was 24.15-49.6 (39.71 \pm 2.65); of head width was 8.65-18.9 (14.91 \pm 1.09); of head length was 6.15- 16.2 (12.82 \pm 1.10); of inter-nasal distance was 2.05-4.4 (3.45 \pm 0.21); of upper eyelid width was 2-4.9 (3.59 \pm 0.28); of interorbital space was 2.9-6 (3.88 \pm 0.33); of distance between back of mandible and nostril was 6.55-12.95 (10.78 \pm 0.70); of distance between back of mandible and eye front was 6.2-11.55 (9.51 \pm 0.54); of distance between back of mandible and back of the eye was 2.55-6.75 (5.05 \pm 0.41); of distance between front of the eyes was 2.45-7.55 (5.60 \pm 0.53); of distance between back of the eyes was 5.8-12.9 (9.17 \pm 0.85); of distance between front of the eye and nostril was 1.75-4.55 (2.60 \pm 0.35); of eye length was 1.45-5.55 (3.08 \pm 0.37); of distance between tip of the snout and nostril was 2.45-4.35 (3.58 \pm 0.20); of distance between front of the eye and tip of the snout was 3.05-7.5 (6.28 \pm 0.52); of greatest tympanum diameter was 1.35-3 (2.45 \pm 0.15); of distance between tympanum and back of the eye was 0.45-2.4 (1.87 \pm 0.23); of forelimb length was 4.65-10.6 (8.44 \pm 0.64); of hand length was 5.75-11.9 (9.17 \pm 0.73); of femur length was 9.05-22.45 (16.29 \pm 1.36); of shank length was 11.35- 18.85 (14.21 \pm 1.59); of tarsus and foot length was 19.5-25 (19.03 \pm 1.75); of foot length was 8.85-21.7 (15.85 \pm 1.44). The factor analysis produced ten variables (correlation 0.80 and above) for 23 morphometric measurements. The two rotated components (D1 and D2) contributed for 85.02% of the variance in the raw data. The first component (D1) contributed for 46.31% of the variability and showed correlation with head length, distance between back of mandible and nostril, distance between back of mandible and front of the eye, eye length, distance between front of the eye and snout tip and forelimb length. The second component (D2) contributed for 38.70% of the variability and was correlated with interorbital space, distance between back of the eyes, hand length and foot length.

EARTHWORM DIVERSITY AMONG VARIOUS LOCALITIES OF MUZAFFARABAD, AZAD JAMMU AND KASHMIR, PAKISTAN

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Earthworms are the most important components of soil biota in terms of soil formation and maintenance of soil structure and fertility. In current study earthworms were collected from different areas of Muzaffarabad, Azad Jammu and Kashmir, Pakistan and their identification was carried out. The samples were collected and four species were identified as *Amyntus minimus*, *Amyntus gracilis*, *Pheretima posthuma* and *Apporectodae rosae* respectively. The study investigated the morphological characteristics like length, color of dorsal and ventral sides, total segments, prostomium shape, age, clitellium segments and shape, and diameter of different earthworm species from Azad Jammu and Kashmir which are not studied before in this area. The distribution of earthworms in different regions is influenced by different soil factors like pH, moisture contents, organic constituents and soil texture. The purpose of present study is to collect and identify the earthworm species from different localities of Azad Jammu and Kashmir which might be helpful to elaborate the importance of soil formation and soil fertility due to existence of earthworms in the field of agriculture and might be helpful for further studies and research on earthworms.

COMPARATIVE ANATOMICAL CHARACTERISTICS OF TWO SYMPATRIC MONGOOSE SPECIES (*HERPESTES JAVANICUS* AND *H. EDWARDSII*) FROM POTHWAR PLATEAU, PAKISTAN

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Two mongoose species reported from Pakistan include the small Indian mongoose (*Herpestes javanicus*) and the grey mongoose (*H. edwardsii*), both are sympatric in many areas of their distribution range in the Pothwar Plateau. Morphologically, the two species differ in their body size, and so it is expected that there could be substantial anatomical differences between them, not yet documented. The current study, therefore, aimed at investigating anatomical characteristics of the two mongoose species inhabiting the Pothwar Plateau. Both mongoose species were live trapped, on monthly basis, from the study area, using especially designed live traps. Poultry intestine was used as bait to attract mongooses. The captured live mongooses were brought to laboratory, and their external body were recorded, after which they were euthanized using chloroform and sacrificed to record data about their anatomical characteristics, including the digestive system, respiratory system, reproductive system, heart, and kidneys and so on. Results showed marked differences between morphological and anatomical characteristics of the two species studied. Morphometrical characteristics like body weight, length of fore limbs, hind limbs and snout length of the two species differed significantly ($p < 0.05$). Among anatomical parameters, liver weight and heart length for females of the two species differed significantly ($p < 0.05$). Similarly, weight of large intestine, stomach, liver and lungs differed significantly between males of the two species. Furthermore, length of heart ($p < .001$) and weight of right and left testes differed significantly ($p < 0.055$) between the two species. Some other variables of the two species that differed non-significantly ($p > 0.05$) included head to tail length, tail length, head length, length of body between head and tail, length of small and large intestines (both in males and females), stomach (males and females), left and right kidneys (males and females) and left and right ovaries, and weight of heart (males and females), right and left kidneys, and right and left ovaries. The study concludes that the two mongoose species (small Indian mongoose and the grey mongoose) show marked morphological and anatomical differences.

ASSESSMENT OF EARTHWORMS BIODIVERSITY IN DISTRICT MIRPUR, AZAD JAMMU & KASHMIR

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Earthworms are soil dwelling invertebrates and enhance the fertility of soil. Present study is a first attempt to investigate earthworm's diversity, their relative abundance, seasonal variations and Shannon-Weiner diversity index in different habitats of the district Mirpur, AJ&K. A total of 12

localities were surveyed and excavated for earthworm from January to December, 2015. A total number of 5684 earthworm specimens were collected from study area representing 10 species of earthworms belonging to 8 genera and 5 families' viz. *Megascolecidae*, *lumbricidae*, *Octochaetidae*, *Moniligastridae* and *Ocnero-drillidae*. *Megascolecidae* (83.16%) was most abundant while *Ocnero-drillidae* (1.86%) least abundant family. *Pheritima posthuma* (37.31%) specie was the most abundant species while *Ocnero-drillus occidentalis* (1.86%) was recorded as rare species. Among habitat, highest Shannon-Weiner diversity index was noted in cropland (H=1.80) followed by garden (H=1.70) while least (H=1.36) index was noted in forest region. Maximum (59.02 earthworms/ft²) was recorded at the altitudinal range of 350-450 m asl which decreased to 12.29 earthworms/ft² above the altitude of 450 m. Maximum richness and diversity were seen in rainy season and in area rich in organic waste respectively. In comparison of the different habitat of District Mirpur, maximum (48%) richness and diversity were in gardens and minimum (5%) in forest plantation. In conclusion physico-chemical factors are highly responsible for the distribution and richness of earthworms in the soil. Such information could be used to take actions for the development of soil to increase agricultural production.

TAXONOMIC STUDIES ON THE HEDGEHOG (GENUS *HEMIECHINUS*) IN DISTRICT JAMSHORO, SINDH, PAKISTAN

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The Hedgehog *Hemiechinus* belongs to the class mammalian, superorder *Laurasiatheria*, order *Eulipotyphla* and family *Erinaceidae*. Hedgehogs (*Erinaceus Europaeus*) are one of the most distinctive and familiar groups of mammals and cosmopolitan in their distribution. Hedgehog is found in xeric desert, especially the acrian plains of Indus River which extends towards east up to Choulistan, Thar and Bahawalpur as well as irrigated Areas of Sindh. The present study was done in order to know the prevalence and variety of the Hedgehog in the different villages as well as city areas of District Jamshoro, Sindh Pakistan. In all, 11 species of hedgehogs were collected from the different localities of District Jamshoro (two species of Hedgehog *Hemiechinus collaris* and *Hemiechinus auritus*) have been identified by the help the prepared keys and available literature. The collected species were alive brings to the Laboratory of vertebrate biology, where Hedgehog were anesthetized with the aid of chloroform, measured with the help of threads and scale, each measurement was taking in mm (millimeter) and further study still carry on to know more characteristics. The present recorded species is considered as new one in District Jamshoro.

SOME OBSERVATIONS ON THE DISTRIBUTION AND ABUNDANCE OF ELAPID SEA SNAKES IN THE COASTAL AND OFFSHORE WATERS OF PAKISTAN

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A total of 15 species of sea snakes 2 genera i.e. *Hydrophis* and *Laticauda* have previously been reported from Pakistan. However, their spatial and temporal distributions have not been fully

described. During the present study it was noticed that 10 species are commonly found along the coast of Pakistan. *Hydrophis viperinus* has been found to be most abundant species in coastal and offshore waters whereas *Hydrophis schistosa* has been mostly encountered in coastal habitats. Study on the spatial distribution indicates that sea snakes are commonly dispersed all along the coastline of Pakistan. In offshore waters of Pakistan, Indus Delta (Khorl Great Bank) along Sindh Coast and area between Malan and Pasni area along Balochistan Coast seem to be a hotspot of sea snakes. *Hydrophis lapemoides* has been observed to be restricted in offshore waters of Sindh whereas *Hydrophis ornatus* and *Hydrophis gracilis* were only reported from Sapat and Ras Malan area. Sea snakes were observed to be found throughout the year, however, maxima of their seasonal distribution were observed during October to December. The paper also revises the taxonomic status of species of sea snakes found in Pakistan.

THE DIVERSITY OF AVIFAUNA IN LAYYAH, PUNJAB, PAKISTAN

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Birds are known as an excellent bio-indicator of the health of an ecosystem. Layyah city is located along the River Indus and is a potential habitat for a variety of resident and migratory species as well. However, there is no record of the diversity and abundance of avian species present in the area. The present study was therefore designed to document the avifaunal diversity and abundance of Layyah City and its surrounding agricultural and riverine area. For this purpose field surveys were carried out from November 2015 to March 2016. Potential habitats around the city were identified and three sites were selected (Site 1=Riverine area; Site 2= Urban area; and Site 3= Cultivated area). Each site was visited twice a month during dawn and dusk and the birds were identified using binoculars and a field guide (Grimmett, 2007). A total of 223 species were recorded from the study area among which 92 were resident species, 87 species were winter migrants, and 42 were year round visitors. A high level of biodiversity was indicated with a Shannon Winner Index of 4.36. Among the recorded species, 16 were observed to be enlisted in IUCN red list as vulnerable, threatened or endangered. The most abundant avian species were House Crow (R.A =13.17), House sparrow (R.A=6.22), Common Myna (R.A=4.55), and Common Starling (R.A=3.59). The highest diversity was recorded in the riverine area ($H' = 4.04$) followed by the cultivated area ($H' = 3.66$) and urban area ($H' = 3.49$). The potential threats to the avian diversity in the area were observed to be hunting, habitat loss, and pollution.

SECTION - VI**POSTER SESSION****OCCURRENCE OF FRUIT FLY *ZAPRIONUS INDIANUS* (DIPTERA: TEPHRITIDAE) IN BER ORCHARDS OF HYDERABAD DISTRICT**

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Zizyus jujuba ber is cultivated all over the drier parts of the Indo-Pak subcontinent for its fresh fruits, which are rich in vitamins and minerals, because of this it is called as apple of the desert. In Pakistan, 'ber' is successfully cultivated in Hyderabad, Khairpur, Multan, Sargoda and Lahore district. Hyderabad district is famous for producing quality fruit which are used locally as well as by exporting we earn foreign exchange. Fruit fly species are the most destructive pest of ber and cause major infestation to ber fruit. Present study Occurrence fruit fly *Zaprionus indianus* carried during ber cropping season 2016 October to March in laboratory and field. Fruit fly *Zaprionus indianus* first time reported from study locality especially from ber fruit, previously this species reported from arid and semi arid region of South Asia, Middle East and China. The emergence of fruit fly started from blossom of ber fruit that is October to November. Females lay eggs on blossom and young fruits. Therefore the fruit got infested and unable for human consumption. Present study revealed that the occurrence of *Zaprionus indianus* first time reported from Sindh in ber fruit. The Second instars larvae were found more active and most destructive stage of fruit fly during whole study period.

**THE STUDY OF DIFFERENT AGROECOLOGICAL ZONES ABOUT JASSID
 (*AMRASCA BIGUTTULABIGUTTULA*) ON BRINJAL
 (*SOLANUM MELONGENA* L.) CROP**

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The abiotic study was conducted to determine spatio temporal trend of jassid on the brinjal crop in the three sites (districts) i.e Faisalabad, Multan and Layyah during 2008. These site are present in the three different agro ecological zones. RCBD was used with the three replication. Data were collected per leaf at weekly based and correlated with the weather factors. The maximum level of jassid were noted in Layyah (4.12/leaf) while minimum population were noted in Multan (3.53/Leaf) and other third site Faisalabad showed the intermediate development of the jassid (3.89/leaf). During statistical analysis simple correlation showed that maximum, minimum and average temperature of all three areas viz Faisalabad, Multan and Layyah showed the positive and highly significant correlation with jassid. The relative humidity of Faisalabad was significant but negative correlation and its rain fall has negative but non significant correlation The Multiple Linear regression showed that maximum temperature has played the key role for the fluctuation of jassid and contributed 53%, 60.9%, 48.7%

and 53% in the fluctuation of jassid in different agro ecological sites of Punjab i.e Faisalabad, Layyah and Multan respectively and also on Cumulative basis. On the Cumulative basis the result showed that the primary role was played by the maximum temperature for jassid population fluctuation i.e. 53.5%, while minimum temperature, average temperature, relative humidity and rain fall had 8.8%, 0.1%, 7.00% and 0.1% role for the population fluctuation.

TRANSFER OF HEAVY METALS IN SOIL, OAT, APHIDS AND BEETLE FOOD CHAIN

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Oat (*Avena sativa*) is easily grown, palatable and nutritious crop that is used in medicine as well as in food of human and milk producing animals. Due to anthropogenic activities, heavy metal concentrations has increased in soil which cause a rise in bioaccumulation of metals in the crop like oat. Heavy metal contents in soil are highly dependent on soil characteristics. Therefore, a study was conducted to analyze physic chemical characteristics of soil under oat cultivation and Zinc (Zn), cadmium (Cd) and lead (Pb) accumulation in plants. The studied soil was alkaline, silt loam in texture and low in soil organic matter. Soil to oat metal bioaccumulation factor (BAF) was above 1 for all studied metals, highest BAF was recorded for Zn (6.14). From oat to aphid, BAF value was above 1 for all Zn and Pb, the highest value was recorded for Zn (2.17). In many sample, BAF value of Cd was less than 1 in aphids. From aphids to beetles, BAF values more than 1 for all metals, highest value was recorded for Zn and least for Cd. Present study highlights the mobility of heavy metals in food chain.

IN VIVO HYPOGLYCEMIC POTENTIAL OF SELECTED MEDICINAL PLANTS AND LACTIC ACID BACTERIA

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Diabetes mellitus is characterized by hyperglycemia that is induced by decreased cellular glucose uptake and metabolism in the body cells. It involves oxidative stress and changes in lipid metabolism. Estimated 84% population of Pakistan depends on traditional medicines and approximately, 450 plants are recorded that have been used in the treatment of diabetes. The majority of plants await proper scientific evaluation for their ability to improve blood glucose. Similarly probiotics have also been used to lower glucose to control diabetes. The current study was conducted to estimate antidiabetic potential of leaves of *Terminalia arjuna* and probiotic strain, *Enterococcus* in *in vivo* model. Moreover, it is a cheap source of herbal medicine and has minimal undesirable effects unlike synthetic drugs. The plant material was extracted with methanol. Female albino mice (n = 35) were divided into 7 groups. Diabetes was induced by alloxan injection (ip) at a dose of 100 mg/kg. Animals of treated groups were given the doses of plant extract (200 mg/kg and 400mg/kg) and probiotics and a group was treated with the combination of both (plant extract i.e. 200 mg /kg and probiotics). The mice were sacrificed following the fourteen days of treatment. Blood glucose level

was recorded on alternative days. Tissues were subjected to histomorphometric studies and serum was analyzed for liver functional test (LFT), renal functional test (RFT), lipid profile and serum glucose level. The methanol extract of leaves *Terminalia arjuna* possessed hypoglycemic activity as shown by decreased fasting blood and serum glucose (66%). Histomorphological examination of pancreas also confirmed antidiabetic potential of plants it was evidenced by normal number of beta cells in plant treated group and group treated with combination of both probiotics and plants. Administration of the extract also improved the lipid profile of the treated groups indicating thereby that the high levels of triglyceride (29%) and total cholesterol (25%). There was also observed no adverse changes in LFT and RFT levels in *Terminalia arjuna* treated animals as well as in animals treated with the combination of probiotics and leaves extract. *Terminalia arjuna* might inhibit glucagon and increase insulin secretion and decrease blood glucose level and further scientific evidences are required to validate its mechanism of action. Glucose reduction was observed in a group treated with combination of *Terminalia arjuna* (200 mg/kg) and probiotics strain, *Enterococcus* and a group treated with *Terminalia arjuna* (400 mg/kg). It showed that there was better hypoglycemic potential with the combined activity of plant extract and probiotics rather than probiotics used in isolation.

EFFECT OF TEMPERATURE AND HUMIDITY ON THE WEB DESIGN OF *NEOSCONA THEISI*

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Spider abundance in a particular area is affected by biotic and abiotic factors. Spiders being ectothermic animals have the ability to alter their web geometry with change in temperature and humidity. Present study was designed to assess the effects of temperature and humidity on web structure of *Neoscona theisi* both in laboratory and field conditions. In field, web parameters of adult female *N. theisi* recorded in summer and winter showed a significant difference in mesh height, capture area, capture thread length and asymmetry. In laboratory, spider exposed to different temperature showed variations in capture area and capture thread length only. No difference was noticed in mesh height and asymmetry of the webs. When the spiders exposed to different humidity treatments, they showed significant difference in capture thread length and capture area, while mesh height, and asymmetry remained similar. It was concluded that environmental factors i.e. temperature and humidity have great impacts on web design of *N. theisi*.

GENETIC HISTORY AND DIVERSITY OF COCKFIGHTING CHICKENS OF PAKISTAN

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The Indian subcontinent cockfighting chickens are famous for their vigour, alertness, fighting behaviour and disease resistances. The origin of the 'breed' is unknown; it might

have been associated to the domestication of the species for recreational purpose. Seals depicting cockfighting, perhaps as old as to 2500 BC have been found at Mohenjo-Daro (Sindh Province). This study aims to understand the origin and diversity of Pakistani cockfighting chicken and to relate this diversity to other indigenous population and commercial birds. Here, we report our results on the mtDNA diversity of 185 birds from all Pakistani cockfighting varieties and 10 captive red jungle fowl (*Gallus gallus murghi*). A 397bp fragment of the mtDNA D-loop was sequenced. Within and between population haplotypes diversity and relationships (neighbor-joining tree and median-joining network) are presented. We address the expected origin and phylogeographic history of the birds with reference to wild red jungle fowl and non-Pakistani chicken populations. Cockfighting chickens have had a substantial influence on the dispersal of the species throughout the world providing a proxy for the understanding of past trading relationships between human communities and civilizations.

IMPORTANCE OF DNA BARCODING TECHNIQUE IN THE IDENTIFICATION AND CONTROL OF ILLEGAL WILDLIFE TRADE

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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. So far we have reported three such cases regarding the illegal trade of different parts of the body and product made from these species. A consignment labeled as "fish meat" intercepted at a Pakistani port 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). In another case, 12 highly processed, chemically treated and finished animal skin (coats, tanned skins) and fur (mufflers) samples were received from the Sindh Wildlife Department, Pakistan, were subjected to DNA mini-barcoding. Eight mufflers belonged to *Vulpes vulpes*, one coat to *Ursus thibetanus*, one tanned skin to *Lutra sumatrana*, and one muffler to *Vulpes* sp. while origin of only one coat sample remained unidentified, showing success rate of 92% of mini barcoding technique for identification of species. Similarly, four highly processed skin samples received from KPK Wildlife Department were identified using mini-barcode primers by targeting 190 bp of COI gene. Two samples belonged to *Gazella bennettii* and two to *Bos taurus*. These reports highlight the problem of smuggling protected species under false pretenses and the importance of DNA barcoding in stopping such illegal trade.

**DIVERSITY OF ADULT HOVERFLIES (DIPTERA: SYRPHIDAE) IN DIFFERENT
HABITATS OF CENTRAL SINDH, PAKISTAN**

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Hoverflies belong to family Syrphidae and comprises of about 6000 species worldwide. They are very important group of insects in agricultural point of view and play major role plant pollination. Diversity of adult hoverfly communities was studied during January to November 2016 in different localities of Central Sindh. Adult hoverflies were collected/trapped by three different methods i.e. Malaise trap, Yellow pan trap and insect hand/sweep net from seven different habitat types. As a result a total of 12 species with 1257 hoverfly individuals were collected, among which *Eristalinus aeneus* (Scopoli), *Episyrphus balteatus* (De Geer) and *Ischiodon scutellaris* (Fabricius) were found to be most abundant whereas, *Eristalinus tanenips* (Wiedemann) was least abundant. Habitat wise variation in species richness, evenness and diversity indices were compared. The highest degrees of species diversity, evenness and richness were recorded in Ornamental flowers where as lowest species diversity, evenness and richness were recorded in fodder crops.

**ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN OVA ALBUMIN
GENE WITH FERTILITY OF CHUKAR (*ALECTORIS CHUKAR*)**

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Chukar (*Alectoris chukar*) the national bird of Pakistan belongs to family Phasianidae. Northern hilly areas of Pakistan are its natural habitat where over hunting and changes in environmental conditions and anthropogenic activities are major threats to its wild population. The ex-situ conservation programmes are initiated at provincial and private sector in the country in order to conserve the bird. There are number of problems associated with such captive breeding programmes among them low fertility rate is one. Number of genes, playing the role in fertility of birds, are found to be mutated thus altering the function and affecting the efficiency of breeding. Ovalbumin gene, expressed in response to sex hormones, is reported to be gene associated with fertility of birds. The present study was designed to assess the association of mutation i.e. SNP's in ovalbumin gene with fertility of A. Chukar. For this purpose, eggshells were used for DNA extraction. The eggshells were categorized into four types i.e. hatched, pricked with dead embryo, unpricked with dead embryo and infertile. The ~ 495 bp fragment of ovalbumin gene was amplified by using newly designed primer pair. The primers gave successful amplification in two categories only i.e. hatched and pricked with dead embryo. The failure of amplification in other two categories is assumed to be due to mutated primer annealing site. The amplified product sequence was further analyzed using in silico tools to determine the possible SNP's in the fragment. Initially, 27 potential SNP's in 495 bp were obtained in which transversions were dominant over transition. NEBcutter tool was used for restriction digestion

analysis. Eight unique enzymes were found for hatched eggshells. Out of these eight, two (CviQI, RsaI) fulfill the criteria for use in PCR RFLP for wet lab confirmation. Online I-TASSER tool was used to predict the polypeptide of amplified fragment. The study predicts the SNP (T → C) associated with fertility and hatchability of chukar eggs at 86th position of amplicon. This pilot study sets the base of association of ovalbumin gene with fertility of chukar and gene can be further explored for possible association with fertility in the bird.

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.

ENVIRONMENTAL ENRICHMENT TOOLS ARE HELPFUL FOR REDUCING THE STRESS IN CAPTIVE ASIATIC BLACK BEARS: A FOCAL SCAN STUDY

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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 Balkasar Bear Sanctuary to study the behavioral changes before and after introducing enrichment tools in six Asiatic black bears (*Ursus thibetanus*). Ethograms were made for active, passive and abnormal behaviors for focal sampling of bears. Wooden logs, cartons and dry grass were used as enrichment structures. Results showed positive behavior with increase in active behavior of bears and there interactions with keepers of sanctuary. Stereotypy was also reduced after application of enrichment tools. Therefore, application of enrichment tools may bring long term changes in behavior of semi captive bears.

ASSESSMENT OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF SNOW LEOPARDS OF GILGIT-BALTISTAN, PAKISTAN USING MOLECULAR TECHNIQUES: A PILOT STUDY

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The snow leopard (*Panthera uncial* syn. *Uncia uncia*) is a large cat native to the mountainous range of Central and South Asia. Under the Conservation Monitoring Centre of the International Union for Conservation of Nature (IUCN), snow leopard has been listed as endangered since 1996. These leopards are threatened due to number of reasons including degradation or loss of habitat, climatic changes, illegal predation, inhuman conflict and poaching for its body parts for traditional medicine. The dedicated efforts are in progress for conservation of this wild cat. The information available on snow leopard population, structure and abundance are so far based on crude field estimates. For effective conservation action plan there is a dire need of advanced laboratory based authentic study. Keeping in view lack of molecular data about the snow leopard in Pakistan, the pilot study is designed which was done by using noninvasive samples of snow leopard collected from different areas of Gilgit-baltistan. The multipronged approach was adapted to optimize and study the genetic diversity of population structures of snow leopard and for this purpose; seven microsatellite markers were used. Total 50 samples were collected from different areas of Gilgit-batistan and samples were identified by the help of field trainers, local villagers and herders. Three methods are used for genomic DNA extraction in order to optimize the best efficient protocol for DNA extraction. Out of 50 samples the DNA was extracted from almost 30 samples in which 25 samples were amplified by seven SSR primers. Data was analyzed by formulating datasheet which was further analyzed by using GenAlex and PopGen software. As expected the genetic diversity was very low which may be due to the seasonal changes and human activities thus leading to the decline in the population of snow leopard. For population structure, sex identification marker were used to find out the sex ratio in which 68% samples having no amplification are of female and 32% samples are identified as male. The optimization of these markers and preliminary study are helpful in providing baseline data for future studies covering complete potential habitats of snow leopard in Pakistan.

**HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN MIDGUT CELLS OF
ANTICARSIA GEMMATALIS (LEPIDOPTERA: NOCTUIDAE)
INDUCED BY SQUAMOCIN**

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Annonaceous acetogenins comprises of a series of natural products which are extracted from Annonaceae species, and among those squamocin proved to be a promising agent. Squamocin is mostly observed as a lethal agent for midgut cells of different insects. It showed toxic effects

when tested on larva of *Anticarsia gemmatilis*. In present study, LC₉₀ and LC₅₀ of squamocin for *A. gemmatilis* were calculated using probit analysis. The Larva was then treated with LC₉₀ and LC₅₀ concentrations of squamocin for 24, 48, and 72 hours, and the ultrastructural changes in midgut were observed under Light and Electron Microscope. Results revealed that the maximum damage to midgut structure was observed under LC₉₀ concentrations where it showed irregularities in labyrinth and lose proximity of basal membrane with columnar cells under light microscope. Moreover, *highly vacuolated cytoplasm, damaged apical surface, cell protrusion* and autophagy in anterior and lumen necrosis in median fragment of the midgut were observed under Transmission Electron Microscope. These results demonstrate that squamocin has potential to induce enough morphological changes in midgut to cause apoptosis and autophagy in cell.

ECOTOURISM DEVELOPMENT FOR SUSTAINABLE MANAGEMENT OF BIOTIC RESOURCES AT MANGLA DAM, AJK

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Mangla Dam of Azad Jammu and Kashmir is deep fresh water wetland spread over an area of 265 Km² and support important biodiversity of region. The present study was planned for to access the sustainable management of biotic resources of this manmade wetland through development of ecotourism. Participatory Human Resource Interaction Appraisals (PHRIA methodology) was applied to access the success of ecotourism development for biotic resource management and biodiversity of the site was recorded by point count method. It was found that study site was rich of biotic resources as floristic composition was consisting of 163 plant species. At site a total of 42 species of fish, amphibians (n=2) and reptiles (n=12) were observed among notable species *Python molurus* was near threatened and *Xenochrophis piscator piscator* was endangered. A total of 57,892 birds belonging to 188 species and 13 species of mammals were identified as well. Among birds and mammals *Sterna acuticauda* and Indian Pangolin are endangered respectively. Mangla wetland contributed to household economy of local people at considerable level as a family of 6 person used 120 kg of wood per month and in this way they burnt 1440 kg of wood annually and promote deforestation. Hunting for subsistence was remarkable at site and total reported cases for illegal hunting were 539 during study period with 14.7 hunting index. Unsustainable fisheries was common at the site a total of 151 cases of illegal hunting of Mahasher and other fishes were reported by means of katra net, electric current and blast fishing. Uprooting of woody trees and livestock grazing at shoreline and buffer was also noticed at the site. The basic causes for these activities were poverty, illiteracy, poor wildlife protection law enforcement, less job opportunities and community hatred. The SWOT analysis was performed and found that ecotourism development can provide alternative way of earning to local community and could reduce pressure on biotic resources of the Mangla dam.

**MOLECULAR IDENTIFICATION OF EGGSHELL MICROFLORA OF CHUKAR
PARTRIDGES (*ALECTORIS CHUKAR*) IN BALKASAR RESEARCH
COMPLEX (DISTRICT CHAKWAL), PAKISTAN**

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Chukar partridges (*Alectoris chukar*) are threatened by plethora of problems because of illegal hunting, poaching, habitat destruction and indiscriminate use of pesticides and herbicides. Many intrinsic and extrinsic factors also affect the mortality in chukar embryo. One of the factors is the microbial contamination of eggshell during incubation, which ultimately effects the embryo and causes hatching failure. So far no study was reported from Pakistan describing the association of microflora with embryo mortality and hatching failure in *Alectoris chukar*. Current study is aimed to isolate inner shell microflora, amplify 16S rRNA gene for bacterial identification and associate their possible effects on embryo mortality and hatchability. A total of 204 eggs were collected from May to August, 2015 from Balkasar Research Complex, Chakwal Pakistan. After completion of incubation period, these eggshells were characterized into four categories i.e., un-pricked with dead embryo, pricked with dead embryo, hatched and unfertilized eggs. A total 47 of 204 (23.03%) samples gave positive results for Gram-positive bacteria and these species were further identified by 16S rRNA sequence analysis. It was found that bacterial colonies on egg shell belong to six different species from genus Bacillus and Staphylococcus, viz., *Bacillus thuringiensis* (4.25%), *B. cereus* (23.40%), *B. toyonensis* (6.38 %), *B. amyloliquefaciens* (17.02 %), *B. subtilis* (38.29 %) and *Staphylococcus sciuri* (10.63 %).

**COMPARATIVE ANALYSIS OF DIFFERENT SPECIES OF GENUS CANIS BY USING
DIFFERENT BIOINFORMATIC TOOLS**

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There are nine different species of Genus Canis distributed in different parts of the world i.e. *Canis lupus*, *C. aureus*, *C. mesomelas*, *C. simensis*, *C. latrans*, *C. lycaon*, *C. adustus*, *C. rufus* and *C. anthus*. However, *C. lupus* and *C. aureus* are found in Pakistan. In the current study, three mitochondrial genes Cytochrome Oxidase subunit I (COI), 16s ribosomal RNA (16s rRNA) and Cytochrome B (Cyt b) sequences were compared to analyze the phylogenetic relationship among extant nine species of genus Canis. The sequences for COI, Cyt b and 16s rRNA of seven species found in different parts of the world were retrieved from NCBI Gene Bank. 16s rRNA revealed *C. latrans*, *C. lycaon* and *C. simensis* shows close phylogenetic relation with each other While COI and

Cyt b showed wider genetic distance. Cyt b and 16s rRNA analysis indicated *C.mesomelas* and *C.adustus* as sister taxa, while COI also proved the same phylogenetic association. COI and 16s rRNA revealed the least genetic distance between *C. aureus* and *C. lycaon* while Cyt b showed more genetic distance. COI and 16s rRNA analysis determined close phylogenetic relations between *C. lupus* and *C. lycaon* while Cyt b showed greater phylogenetic distinction between these two species. Cyt b and 16s rRNA revealed no genetic distance between *C.latrans* and *C.lycaons* while COI analysis identified both as genetically distant. Finally, COI, 16s rRNA successfully determined the phylogenetic association between *C. aureus* and *C. adustus* but Cyt b clarified more with higher genetic distance. Hence it is concluded that 16s rRNA revealed least genetic distance while Cyt b showed higher genetic distances which suggest its potential use for species/sub-species delineation especially for mammal.

HISTOPATHOLOGICAL CHANGES IN THE ABOMASA OF INDIGENOUS GOAT BREEDS TOWARDS EXPERIMENTAL INFECTION OF *HAEMONCHUS CONTORTUS*

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Abomasum is one of the most important site for living bursate nematodes belonging to family Trichostrongylidae in goats. Three pathogenic species of GI nematodes viz *Haemonchus* spp. *Ostertagia* spp. and *Trichostrongylus* spp. *Haemonchus* (*H.*) *contortus* is a predominant, highly pathogenic and economically important nematode parasite of goats round the globe. The disease caused by this nasty parasite is known as haemonchosis. Control of this parasite is complicated by the presence of widespread resistance to the available anthelmintic groups. Identification of parasite resistance goat breed may be a significant approach to control this nuisance. Histopathological changes in the abomasa of goats exposed to experimental infection of *H. contortus* may be a significant indication of resistance/ susceptibility status of goats. In this prospect, a research project has been initiated by Department of Parasitology, University of Agriculture, Faisalabad with the aim to evaluate indigenous goat breeds (Beetal, Teddy and Dera Din Panah) for their natural resistance towards *H. contortus*. Abomasal and intestinal tissues of artificially infected animals has been evaluated through histopathological changes. Preliminary results of present study have indicated that DDP goat breed showed presence of clear histopathological changes compared to Beetal and Teddy goats. Through histopathological evaluation, present study has depicted the variability in response of goat breeds towards haemonchosis specifically and parasitism in general. This indication of breed resistance towards parasitic infections will provide the guidelines in selective breeding of disease resistant livestock in Pakistan, which is an indirect way to control the parasite related losses.

PROCUREMENT AND CHARACTERIZATION OF CRUDE SOMATIC AND EXCRETORY SECRETORY ANTIGEN OF *HAEMONCHUS CONTORTUS* INFECTING THE DOMESTIC GOAT BREEDS OF METROPOLITAN FAISALABAD

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Haemonchosis is a disease caused by an abomasal parasite *Haemonchus (H.) contortus*. Antigen based diagnosis of haemonchosis can play significant role in the detection of subclinical disease. In the present research, crude somatic (CS) and excretory secretory (ES) antigen was produced from different goat breeds slaughtered at Faisalabad slaughter house. The adult worms were collected from abomasum and washing was done through standard protocols. Morphological identification of worms was carried out through standard keys and incubation was carried out at 37°C for 24 hours. For procurement of CS antigen worms were collected and washed with phosphate buffer saline. The worms were then transected and homogenized in ultrasonic tissue homogenizer followed by refrigeration for 8 hours. After centrifugation, the supernatant was stored as crude solubilized antigen. Characterization of CS and ES antigen was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and protein concentration estimation. The distinct protein bands were observed for ES and CS antigens of *H. contortus*. The molecular weight of CS antigen of *H. contortus* were 48, 60 and 65 kDa with three distinguished proteins. The ES Ag *H. contortus* was equal to CS Ag which was of 65 and 48kDa whereas ES Ag showed an additional band of 30 kDa that was an exclusive protein moiety. Results of this study can be further helpful in evaluation of immune response of CS and ES antigen against *H. contortus* in goats.

FREQUENCY DISTRIBUTION OF GASTROINTESTINAL PROTOZOA AMONG HOME BRED AND DAIRY CALF POPULATION OF DISTRICT KHANEWAL, PAKISTAN

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Gastrointestinal (GI) protozoal infections, particularly Giardiasis and Cryptosporidiosis pose a serious health threat to young dairy calves through high morbidity, mortality and treatment cost in developing countries like Pakistan. In the present study, a passive surveillance was conducted for period of six month from January, 2016 to June 2016. Briefly, faecal samples were collected directly from the rectum of young calves on monthly basis from different Dairy Farms, Veterinary Hospitals and small farmers in Khanewal district. Information about some intrinsic and extrinsic factors associated with GI protozoal infections of young dairy calves were collected on a pre-designed questionnaire containing closed ended questions. Collected faecal samples were preserved in 10% formal saline and transported to Epidemiology Laboratory, Department of Parasitology, University of Agriculture, Faisalabad. These faecal samples were further subjected to

microscopic examinations using standard protocols. The prevalence of Cryptosporidiosis and Giardiasis was detected by Modified Ziehl Neelsen (acid fast staining) and Zinc Sulfate Flotation technique, respectively. Overall prevalence of cryptosporidiosis and giardiasis was recorded as 49.32% and 31.23% respectively. Age, sex, breed, late colostrum feeding and mix faring system were found statistically significant ($P < 0.05$) in both cases cryptosporidiosis and giardiasis while floor pattern and housing system were not statistically significant ($P > 0.05$). Collected data can be used to develop some control strategies that will indirectly help the farmers to improve health and production of dairy calves.

ESTIMATION OF PLASMA HISTAMINE LEVELS IN THREE INDIGENOUS GOAT BREEDS OF PUNJAB, PAKISTAN EXPOSED TO CHALLENGED INFECTION OF *HAEMONCHUS CONTORTUS*

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Selective breeding of resistant goat is a principle strategy to control gastrointestinal (GI) nematode parasites. The current study was scheduled to ascertain the response of Beetal, Teddy and Dera Din Panah (DDP) goat breeds against challenged infections using a candidate nematode; *Haemonchus (H.) contortus*. A total of 72 goats (24 from each breed) were purchased, housed, acclimatized, grouped out randomly and screened for the Faecal Egg Count. Infective third stage larvae (L₃) of *H. contortus* were harvested through coproculture to induce artificial infection. Goats that were free from any GI infection were further exposed to artificial infection challenge with L₃ larvae. Blood samples were collected for plasma separation and further used for the determination of plasma histamine. Experimental goats were kept in controlled environment throughout the experiment. Total histamine concentration in plasma was measured through histamine determination kit (Labor diagnostika Nord GmbH & Co. KG, Germany) according to the manufacturer's protocol. Histamine concentration was persistent during the course of infection and significant ($P < 0.05$) association was found with resistance of breeds towards *H. contortus* infection challenge. Overall, low concentration of plasma histamine level depicted that Teddy breed of goat is most resistant to GI parasites followed by Beetal and DDP. In conclusion, three different goat breeds showed different response towards *H. contortus* infection. Ultimately, this variation in response will formulate the base of selective breeding of resistant goat breeds to ensure the sustainable goat production in the country.

FLUORIDE AND BIFENTHRIN HISTOPATHOLOGY OF EYE IN DEVELOPING CHICK

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Developmental alterations of embryonic eye on in ovo exposure of F and Bifenthrin (a fluoridated insecticide) were explored in golden black variety of domestic chick. Fertilized 165

eggs were equally distributed (n=55) in three groups called – Fluoride (received inter-vitelline injection of 0.01 μ g/g fluoride ions from NaF in 0.1ml of 5% DMSO aqueous solution on 0day of the study); Bifenthrin (received inter-vitelline injection of 0.01 μ g/g technical grade Bifenthrin in 0.1ml of 5% DMSO aqueous solution) and Vehicle group (received inter-vitelline injection of vehicle i.e. 5% DMSO aqueous solution). The eggs afterwards were incubated at 37 \pm 0.5 $^{\circ}$ C under 65% humidity for 14 days. All embryos were externalized out of the embryonic membranes and egg shell on day15. All embryos were fixed in 10% alcoholic formaldehyde for 48 hours before they were dissected to remove intact eye balls from the embryonic optic sockets. Each eye was weighed and finally processed for micro-anatomical, micrometric and histopathological studies. The histological sections have shown denser and enlarged marginal mitotic region of the developing eye lenses in fluoride and bifenthrin as compared to the vehicle group. In vertical sections of the eye lenses the nuclei of the crystalline cells in F and Bifenthrin groups show a highly depressed bow shaped arrangement than the vehicle group. Moreover, the nuclei of the core crystalline cells of the lens were apparently smaller in F and Bifenthrin groups than the Vehicle group. Out of the six anatomical layers of the retina the nuclear and the plexiform layers were highly enlarged in F and Bifenthrin groups, similarly the three corneal cell layers (endothelium, parenchymal and the epithelial) were enlarged in F and Bifenthrin than the vehicle group. Analysis of the morphometric data has revealed highly significant decrease ($p \leq 0.001$) in mean weight (VC=233.31+7.42mg, F=169.4+1.05mg, BF=118.26+8.64mg) and density (VC=0.9628+0.05mg/ μ l, F=0.64+0.009 mg/ μ l, BF=0.46+0.002 mg/ μ l) of the eye balls in F and BF groups. Similar highly significant decrease in mean cell density of lens/100 μ was observed (VC=1413.56+50.73, F=954.50+88.87, BF=777.56+39.79). The findings indicate that fluoride is a potent disruptor of avian eye development both in ionic and organic forms.

PREVALENCE OF APHIDOPHAGOUS HOVERFLIES IN RELATION TO THEIR PREY, GREEN APHIDS (*MYZUS PERSICAE*) ON BRASSICA (*BRASSICA RAPA*) IN DADU

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Dadu is one of the main regions of Sindh, Pakistan with respect to the agriculture, in which different crops are grown throughout the year due to its moderate environmental condition and quality of soil such as brassica, spinach, wheat, tomato, rice. These crops are badly attacked by different crop pest including aphids which are polyphagous and causing damage to many crops. Aphidophagous hoverflies are one of the important group of insects belonging to order diptera family syrphidae, subfamily syrphinae. Worldwide about 6000 species have been reported so far, the larvae of most of the species subfamily syrphinae are predator of aphid and jassid. These pests destroy many crops like brassica, spinach, wheat, tomato etc. by sucking their sap, which affects the growth of plant and also the production of crop. The study was conducted from August to November 2016 in Dadu and its adjoining areas in which different fields were visited during winter season, ecological factors and host plants were also noted. Total 211 specimens of 3 species (*Eupeodus luniger*, *Ichizodon scutelleris*, *Episyrphus balteatus*,) belonging to genus *eupeodus*, *ichizodon* and *episyphus*, respectively, family syrphini were collected on different host plants; 77 specimens of *Ichizodon scutelleris*, 44 *eupeodus* and 90 *Episyrphus balteatus*. These species were identified as aphidophagous present in the colonies of green aphids (*Myzus persicae*) on brassica (*brassica rapa oleifera*).

**EFFECTS OF ANESTHETIC COMBINATION OF MEDITOMIDINE-
KETAMINE IN ARABIAN STRIPED HYAENA
(*HYAENA HYAENA SULTANA*) ON VARIOUS PHYSIOLOGICAL
PARAMETERS AT SIR BANI YAS ISLAND**

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Chemical capture is typically known to involve use of anesthetic drugs to restraint or immobilize animals for different conservation or management purposes. The process involves delivery of the drug in dart through any projection system, *e.g.* jabstick, blowpipe or rifle. Conservationists, wildlife managers, veterinarians, animal welfare personnel as well as zoo workers face great deal of difficulty when it comes to the safe (both to animal and human) handling and restraint of potentially dangerous or animals' sensitive to capture myopathy. Chemical capture of animals has advanced as an essential tool in wildlife management over the past few years. The Arabian striped hyaenas (*Hyaena hyaena sultana*) are nocturnal carnivores of Hyenidae family with five subspecies based on geographical variations and are listed as near threatened (NT) under IUCN Red List of Threatened Species. Their distribution range is Oman, Saudi Arabia, United Arab Emirates and Yemen. There is no confirmed record of *H. h. sultana* in United Arab Emirates in recent years. However, Ex-situ conservation efforts are ongoing in various conservation and zoological institutions. With years of dedication and conservation efforts, Sir Bani Yas Island hosts more than 16000 animals (from 4 species of carnivores, 18 species of ungulates, 3 ratites, and 2 reptiles) and over 2.5 million trees. Sir Bani Yas Island has 16 individuals of *H. h. sultana*. There is very little to no thorough studies available on this sub species. A Ph. D study was conducted on a total of 37 hyaena that were immobilized with a combination of 0.04 mg/kg of Medetomidine hydrochloride (1mg/ml) and 2.2 mg/kg of Ketamine hydrochloride (100mg/kg). The average time to full anesthesia was 11.64±8.12 minutes. Atipamezole hydrochloride was used as reversal with the dosage of 6.5 mg/kg. The average reversal time was 6.14 minutes. The average temperature was recorded as 99.85±1.5 °F, the average pulse rate was 43.42±3.75 per minute, the average respiration was recorded as 31.40±2.34 per minute, and average spO₂ was recorded as 81.71±16.93. The animals went into deep anesthesia to allow various procedures to be performed and the recovery was smooth.

HABITAT AND FOOD PREFERENCES OF GREY FRANCOLIN (*FRANCOLINUS PONDICERIANUS*) AND BLACK FRANCOLIN (*FRANCOLINUS FRANCOLINUS*) IN RAKH SARDARAN GAME RESERVE, DISTRICT HARIPUR, KHYBER PAKHTUNKHAWA, PAKISTAN

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Grey francolin (*Francolinus pondicerianus*) and Black francolin (*Francolinus francolinus*) are the key game birds of Rakh Sardaran Game Reserve in District Haripur. The present study was conducted to find out the comparative population density, habitat preference and diet composition of both species in Rakh Sardaran Game Reserve. The study area was divided into four sub-study sites, representing all the types of major habitat. Line transect method was used to study the habitat preference in the selected sites and the relationship between habitat and francolin numbers was also counted for all the study sites. The results showed that the overall population density of Grey and Black francolin was 2.4birds/km² and 1.5birds/km² respectively. The habitats of rocky area support the highest population density i.e. 0.6birds/km² of Black francolin followed by plain areas i.e.0.5birds/km², stream beds, 0.1bird/km², and agricultural land 0.3birds/km². Highest density of Grey francolin was observed in rocky area i.e. 0.9birds/km² followed by plain areas, 0.7 birds/km², agricultural land, 0.5birds/km² and stream beds, 0.3birds/km². Crop and gizzard were collected during the hunting season for diet analysis of francolin species. Grey francolin diet comprised of plants (23%), seed (38%), insects (21%) and others (18%). Black francolin preferred to feed on plants (43%), seed (33%), insects (19%) and others (5%). Food preference revealed similarity in types of food consumed with slight variation in preferred diet at different study sites. Floral and faunal variety of food contents demonstrated the omnivorous and food generalist characters of francolin species. Habitat preference showed both sympatric species shared all the types of habitat with small variation in during the breeding season. They preferred to live in separate population patches within the same habitat. The major threats included illegal hunting, poaching, overgrazing, use of pesticides and fertilizers in the study area. There is dire need to evaluate agricultural practices and the effectiveness of environmental conservation measures. The baseline study will be helpful to devise better conservation strategies of game birds of Rakh Sardaran Game Reserve, District Haripur to enhance the conservation efforts.

OIL DETERMINATION OF MICROALGAL CELLS BY NILE RED STAINING GROWN ON AGRICULTURAL WASTE

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Due to the continuous increase in global energy demands and rapid depletion of fossil fuels in the past few years, need is to exploit sustainable and renewable resources for energy generation.

As a result of gradual depletion of fossil fuels and emission of green house gases (GHG), microalgae are being used as non-food feed stocks for biodiesel production. Nile red staining is a diagnostic tool to measure the neutral lipids that the cells accumulate, as neutral lipids are the feedstock for biodiesel. In the present study two freshwater and one marine species were grown mixotrophically on bagasse. Oil accumulation was investigated with the help of Nile red staining. It was found out that microalgae grown mixotrophically have more oil droplets as compared to the ones grown autotrophically. This sustainable technology of using waste as substrate for microalgae growth not only helps in dealing with the waste/residue being generated but also in release of considerable amount of O₂ to the atmosphere which also helps in coping with the issue of GHG emissions.

**GENETIC SELECTION OF PARASITE RESISTANT GOAT BREEDS: A STEP
TOWARDS SUSTAINABLE PARASITE CONTROL**

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Gastrointestinal (GI) parasitism remains a major constraint associated with the production of small ruminants under grazing/browsing conditions. The GI parasitism control strategies that usually adapted are chemotherapy, vaccination, pasture exposure, ethnoveterinary practices, pasture and grazing management, but all these have their own limitations such as anthelmintic resistance (AR), drug residues, cost of purchase, efficacy and environmental concerns. Genetic selection of lines or breeds of hosts (e.g goats) is a complementary tool used to control GI parasitism. Globally, studies have shown variation in response of indigenous breeds of goats towards their resistance against GI parasites. Documentation and selection of these breeds will formulate the base of selective breeding of resistant goat breeds. Selected breeding of resistant breed in the area will enhance the economy of the herd owners in terms of negligible parasitic infections, cutting off treatment cost, low morbidity/mortality and high production. In this prospect, a research project has been initiated by Department of Parasitology, UAF with the aim to evaluate indigenous goat breeds (Beetal, Teddy and Dera Din Panah) for their natural resistance towards GI parasitism. Preliminary results of present study have indicated that Teddy breed of goat is showing some inherent resistance towards parasites. In this regard, it may be recommended that selective breeding of parasite resistant goats should be carried out at rural as well as commercial level. Selectively bred animals will be more productive and disease resistant. This practice will not allow the establishment of disease and consequently may reduce the use of anthelmintics. Also, this will ensure availability of chemical free meat and milk for human consumption. Ultimately, this effort will be a way forward to achieve the goal of secure food for human consumption.

ASSEMBLAGE AND DIVERSITY OF FINFISH FAUNA COLLECTED THROUGH GILL NET FROM SONMIANI BAY, BALUCHISTAN, PAKISTAN

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The Sonmiani Bay Lagoon (Miani Hor) is mostly a sandy mangrove creek area possess a huge variety in biomass diversity, abundance and species richness. The finfish fauna were sampled by two types of gill net (Nylon and Silk) between 2001 and 2002 to examine the species assemblage and diversity from two offshore areas (Damb and Bhaira) Sonmiani Bay, Baluchistan, Pakistan. The 54 finfish species that represent about 24 families were captured by nylon gill net in off Damb mangrove area. Mugillidae (28.88%), Leiognathidae (17.18%) and Engraulidae (16.47%) were observed three most abundant families. The 18 families comprise about 37 species were captured by silk gill net in off Damb mangrove area and three most abundant families includes Clupeidae (19.55%), Leiognathidae (16.20%) and Mugillidae (13.97%). Total 16 families were captured by nylon gill net in Bhaira mangrove area comprising 42 species and three most abundant families Engraulidae (31.76%), Carangidae (15.88%) and Clupeidae (15.29%) were observed. Engraulidae (37.84%), Mugilidae (15.32%) and Clupeidae (13.51%) were observed three most abundant families out of 16 families were captured by silk gill net in Bhaira mangrove area which comprise 36 species of finfish. The common finfish species include, *Liza carinata*, *L. subviridis*, *Valamugil speigleri*, *Leiognathus blochi*, *Secutor insidiator*, *Hilsa Kelee*, *Ilisha megaloptera*, *Scomberoides commersonianus* and *Thryssa mystax* were recorded from gill net. The significant variability ($p < 0.05$) was observed in diversity and equitability between the sites and types of gill net, however, highest in nylon gill net in the Damb area, but there were no significant seasonal differences in finfish fauna.

A STUDY ON EFFECTS OF TITANIUM DIOXIDE AND TITANIUM DIOXIDE NANO PARTICLES ON GONADS OF MALE SPRAGUE DAWLEY RATS AND ANTAGONISTIC EFFECTS ON *CINNAMOMUM CASSIA* AND *AZADIRICHTA INDICA* EXTRACT

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This study investigates the toxic effects of TiO₂ and TiO₂ nanoparticles and the ameliorating effects of *Cinnamomum cassia* and *Azadirachta indica* on gonads of albino rats. Rats were injected subcutaneously with TiO₂ and TiO₂ 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, hematology, micronuclei assay and testicular histology were investigated at start of experiment and 28 days after treatment. TiO₂ and TiO₂ nanoparticles exposed groups showed significant pathological variation in testies such as reduction in sperm production, apoptosis inflammatory cells, elongation of cells, dysfunctioning of somniferous tubules, and low down process of spermatogenesis. In 2nd phase of experiment subcutaneous injection of TiO₂ or TiO₂ nanoparticles at dose of 150mg/kg body weight of rats for 28 days along with oral administration of cinnamon or

neem extract or both in combine showed that plant extracts at dose of 100 or 150mg/kg reduced the toxic effects of TiO₂ and TiO₂ NPs which is evident by reduced alteration in testicular pathology and decreased sperm damage. So, it is concluded that *Cinnamomum cassia* and *Azadirachta indica* showed remedial or healing effects in rats exposed to TiO₂ sub-lethal dose as these plants are known for their anti-oxidative properties.

MONITORING THE POPULATION OF PULMONATES FROM DIFFERENT HABITATS OF WHEAT IN DISTRICT FAISALABAD

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Land snails are the second largest group of invertebrates that belong to the class Pulmonata of class Gastropoda. More than 100,000 species of land snails are reported and they contribute six percent in the total species of earth. The current study focused on abundance and species composition of land snails in the wheat fields from Pensra and Bhowana of jhang site (North, East) and from Makkowana and Awagatt of Jaranwala site (North, East) in district Faisalabad, Punjab Pakistan. Sampling was done from the wheat and each selected study field was an area of 2.5 acres divided in to 4 quadrates. Physical parameters of soil (moisture, lime content and pH) and Morphometric characters of snails (height, diameter, umbilicus diameter, number of coils, height of spire and other morphometric considerations of shell) were also taken. A total of 695 specimens were collected and representing 6 families, 7 genera and 7 species of phylum mollusca. Shannon diversity Index on the diversity and abundance of land snails population in both fields showed the value of (94.8%) for diversity and dominance value is 0.1793 (17.9%). The statistical Analysis on comparison of both selected fields of wheat did not show any significant result having T value of (0.630) and P value (0.518) but the Co-relation and regression on the monthly variation showed significant result in first two months (November and December) having (P<0.018) and (P<0.030) respectively and rest of months showed non-significant results. Statistical analysis of T test on Soil composition in relation with the land snail population did not show any significant difference among both fields.

HOST PLANT RANGE OF APHIDOPHAGUS HOVER FLIES IN RELATION TO THEIR PRAY APHIDS IN MIRPUR KHAS

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Hover flies commonly known as flower flies belong to large family of small to large flies worldwide about 6000 species have been reported, hover flies provide crucial ecosystem services as pollinators, biological control agent. Aphidophagous hover flies are one of the important group of hoverflies because their larvae feed on many insect pest i.e. aphids. Jassids, thrips, which feed on several crops like brassica, spinach, rice, wheat etc. present

study help in pest management of crops in Mirpurkhas and its peripheral areas. Mirpurkhas is the main agricultural area of Sindh where different flowering crops and fruits are produced such as Mango, Banana, Guava, Brassica, Wheat, and vegetables. The present study was conducted from October to, December 2016 from Mirpurkhas in which different fields were visited during seasonal crops. Total 252 specimens of 3 species (*Ichizodon scutelleris*, *Episyrphus balteatus*, *Bacha baltetus*) belonging to genus *Ichizodon*, *Episyrphus* and *Bacha* family syrphinae were collected from brassica, spinach and chili paper mostly in aphid colonies.

**PREVALENCE OF APHIDOPHAGOUS HOVERFLIES IN RELATION TO THEIR PREY,
GREEN APHIDS (*MYZUS PERSICAE*) ON BRASSICA (*BRASSICA RAPA*) IN DADU**

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Dadu is one of the main regions of Sindh, Pakistan with respect to the agriculture, in which different crops are grown throughout the year due to its moderate environmental condition and quality of soil such as brassica, spinach, wheat, tomato, rice. These crops are badly attacked by different crop pest including aphids which are polyphagous and causing damage to many crops. Aphidophagous hoverflies are one of the important group of insects belonging to order diptera family syrphidae, subfamily syrphinae. Worldwide about 6000 species have been reported so far, the larvae of most of the species subfamily syrphinae are predator of aphid and jassid. These pests destroy many crops like brassica, spinach, wheat, tomato etc. By sucking their sap, which affects the growth of plant and also the production of crop. The study was conducted from August to November 2016 in Dadu and its adjoining areas in which different fields were visited during winter season, ecological factors and host plants were also noted. Total 211 specimens of 3 species (*Eupeodus luniger*, *Ichizodon scutelleris*, *Episyrphus balteatus*,) belonging to genus *eupeodus*, *ichizodon* and *episyphus*, respectively, family syrphini were collected on different host plants; 77 specimens of *Ichizodon scutelleris*, 44 *eupeodus* and 90 *Episyrphus balteatus*. These species were identified as aphidophagous present in the colonies of green aphids (*Myzus persicae*) on brassica (*brassica rapa oleifera*)

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST SOIL ISOLATES

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Essential oils also known as the volatile oils which are extracted from the different parts of the plants such as bark, flowers, buds, leaves, fruits, seeds, wood and roots through many kinds of extraction techniques. Essential oils of plants and herbs have been practiced since ancient times for therapeutic purposes, as flavouring and preservative agent as an alternative to medicines. In present days to minimize or cope with the alarming situation

caused by the bacterial resistance the need to develop potential natural antimicrobials has increased to use as a substitute. Due to wide applications of essential oils the need to practice them and explore their potential has increased. The present study was therefore conducted to evaluate the antimicrobial potential of essential oils including Cinnamon (*Cinnamomum verum*), Basil (*Ocimum basilicum*), and Clove (*Syzygium aromaticum*). For this purpose ten rhizospheric bacteria were isolated from fifteen soil samples of nodular plants. All isolates identified as Gram positive by morphological identification. Biochemical tests (Catalase, Methyl red test, Carbohydrate fermentation, Nitrate reduction) were performed. Bacterial isolates were identified as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cerus*, *Pseudomonas fluorescense* and *Escherichia coli*. Antimicrobial effect checked by Agar well diffusion and Disc diffusion methods by using different concentrations of three essential oils. Antibacterial activity evidenced by the inhibition zones around the discs or wells. All the essential oils showed inhibitory effect to tested bacteria at varying levels. In agar well method maximum zone of inhibition observed against SI4 (*Staphylococcus aureus*) isolate with mean of inhibition zone diameter observed as 12.7 ± 1.4 while minimum observed against SI9 (*Escherichia coli*) with 1.4 ± 0.6 that showed its susceptibility to essential oils. Cinnamon oil exhibited the highest activity. The order of their activity observed as *Cinnamomum verum* < *Ocimum basilicum* < *Syzygium aromaticum*. In disc diffusion method maximum zone of inhibition recorded as 11.7 ± 1.5 and minimum as 0.7 ± 0.6 . At the 1:20 ratio all three of the essential oils cinnamon (11.7 ± 1.5), clove (9.3 ± 2.0) and basil (8 ± 1.9) showed its maximum activity against SI5 (*Staphylococcus aureus*). The overall significant result found by agar well diffusion assay with maximum activity of essential oils recorded at 1/2, 1/4 and 1/8 dilutions while in disc diffusion method maximum antimicrobial potential observed at 1:20.

**ISOLATION AND CHARACTERIZATION OF ANTIMICROBIAL COMPOUND FROM
PAAN LEAF AND ITS POTENTIAL ROLE IN FORMULATION IN
PREPARATION OF MOUTH WASH**

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Paan is considered as a part of cultural heritage of Asia. It was used by Mughal emperors as well. It is cheap and easy to access for public. In this research work, Gram positive cocci (*Staphylococcus*, *Streptococcus* species) were isolated from dental samples collected from healthy individuals and patients suffering from dental problems. The extract of paan leaf was prepared in ethanol, methanol, chloroform and water in different concentrations (100%, 50%, 25%, 10%). Clove was used as a control. Ethanol and methanol extracts showed antimicrobial activity. HPLC revealed quercetin, gallic acid, vanillic acid, chlorogenic acid, m-coumeric acid, cinamic acid and benzoic acid. Among these phytochemicals, quercetin and benzoic acid are known to inhibit microbial growth. These will be isolated, purified and their antimicrobial activities will be checked. A formulation will be prepared which can be used as a mouth wash.

CHARACTERIZATION OF THERMOSTABLE LIPASE FROM *STREPTOCOCCUS* SPECIES AND ITS POTENTIAL APPLICATION IN INDUSTRY

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Lipase is an enzyme famous for breaking lipids. It has vast applications in variety of industries like pharmaceuticals, textile, food, etc. In this research work, from ten samples were collected from contaminated environment to isolate lipase producing bacteria, six bacterial isolates were obtained. Basic lipase screening methods confirmed lipase production by all six isolates. On the basis of lipase quantification, SM-2 showed maximum lipase activity. It was found to be *Streptococcus* by Gram staining and biochemical tests. Olive oil was found to induce the growth of lipase at pH 7 and 37°C. Lipase was isolated from SM-2 and partially purified by using 80 % ammonium sulphate followed by dialysis. The dialysate was run through Sephadex-100 column and DEAE column. The partially purified enzyme showed maximum activity at 50°C, pH 7 and in the presence of Mg⁺² and Cu⁺² ions but less with Zn⁺² and Ca⁺² ions. The band appeared on thin layer chromatography was confirmed by SDS-PAGE which revealed its size of approx. 50kDa. The structure of lipase will be determined by NMR. Ribotyping will help to identify the bacterial isolate. Finally, the isolated enzyme will be checked for its potential application in industry.

THE EXTANT DIVERSITY OF HERMIT CRABS OF KARACHI COAST CONFIRMED THROUGH SDS PAGE ELECTROPHORESIS (PRELIMINARY STUDY)ALTAF HUSSAIN NARIJO,^{1,*} NOOR US SAHER¹ AND MUSTAFA KAMAL²¹*Centre of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan*²*Department of Biotechnology, University of Karachi, Karachi, Pakistan**Corresponding Author: noorusaheer@yahoo.com

Anomura (Decapoda, Crustacea) family Paguridae commonly known as Hermit crabs. The anomurans are one of the most morphologically and ecologically diverse groups of decapod crustaceans, including a large number of extant species. The present study is based on the diversity of Hermit crab, initially confirmed through SDS page and native page electrophoresis. During this study the hermit crabs collected from five sites (Sonmiani bay, Sea view, Sonari beach, Korangi and Russian beach) along the coast of Pakistan. The collected crabs belong to three genera *Clibanarius*, *Diogenes* and *Calcinus* of family Diogenidae. The individual species were varied according to size and the larger one individual (size: TL: 9.8 cm, SL: 1.4 cm, Chela: 4.4 cm) belongs to *Clibanarius infraspinus* species and the smallest one (size: TL: 2.5 cm, SL: 0.3 cm, Chela: 01 cm), which is *Calcinus lateens* comparing with shell aperture. The SDS gel electrophoresis were applied for the preliminary biochemical differentiation of collected and identified species and confirmed after molecular identification.

**IDENTIFICATION OF miRNAs ASSOCIATED WITH ENDOMETRIOSIS
IN PAKISTANI WOMEN****Threem Zia*, Irfana Liaqat* and Hina Dilber Khan***Department of Zoology, GC University Lahore, Pakistan**Corresponding Author: irfana.liaqat@gcu.edu.pk

Endometriosis is a gynecological condition that affects the female reproductive system and ultimately causes infertility. This disease is present worldwide in all racial groups. Incapacitating signs and symptoms with global prevalence were the reasons of unrevealed pathogenic mechanism of endometriosis. Various risky health phenomenon in relation to endometriosis may triggered by plasma micro RNA (miRNAs) which possibly, could be changed by therapeutic techniques used for endometriosis. For early diagnosis of endometriosis, the miRNAs role as biomarker is crucial. The blood samples of genetically unrelated endometriosis women and controls, 80 each, were used to isolate RNA. This total RNA was used to synthesize cDNA. This cDNA was used to identify miRNAs either down regulated or up regulated for further confirmation of endometriosis by real time PCR. Twenty microRNAs were differentially expressed in women with endometriosis and their controls. Out of which six microRNAs (miR-15a, miR-15b, miR-16-1, miR-16-2, miR-195 and miR-497) were selected for validation. MiR-15a, miR-15b and miR-195 were significantly down-regulated in women with endometriosis when compared with controls ($p= 0.011, 0.002$ and 0.002 , respectively), yielding an area under the receiver operator characteristics curve of 0.74 (95% CI: 0.58–0.90; 0.79 (95% CI: 0.65–0.93) and 0.85 (95% CI: 0.71–0.98) in endometriosis from controls respectively. miRNAs appear to be potent regulators of gene expression in endometriosis and its associated reproductive disorders, raising the prospect of using miRNAs as biomarkers and therapeutic tools in endometriosis. Identification of miRNAs lessens the health care cost and improves the work productivity of endometriotic females.

**ISOLATION AND CHARACTERIZATION OF TANNASE PRODUCING BACTERIA
FROM LOCAL ENVIRONMENT****WAJIHA KANWAL AND ABDUL REHMAN***Department of Microbiology and Molecular Genetics, University of the Punjab,
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Microbial enzymes are most promising catalysts in industrial biotechnology due to their striking properties. In the present study, four bacterial isolates designated as WK-1, WK-A2, WK-A3 and WK-4 showed the maximum tannase activity and were selected for further study. The optimum growth temperature for WK-A2, WK-A3 and WK-4 was found to be 30°C while it was 37°C for WK-1. Optimum pH for growth of WK-1, WK-A2 and WK-A3 was found to be 5, 6 and 10, respectively. WK-4 was found to be able to grow effectively on acidic as well as basic pH. Growth curves of these organisms showed that growth was slower in the presence of tannic acid. In controlled environment (N-broth), organisms showed a shorter lag phase and reached at log phase within 4-12 h. Tannase activity can be cell associated or extracellular. The enzymes produced by WK-4 showed highest activity at 45°C. Tannase produced by WK-A2 and WK-A3 was found to be stable within temperature range of 30-45°C. Tannase from WK-1 was fairly active at all temperatures as indicated by its activity between 25-45°C, however, the activity started to decline after 45°C. Optimum enzyme pH for WK-1, WK-A2 and WK-4 was found to be 6 while WK-A3

exhibited maximum activity at pH 8. The tannase activity was enhanced in the presence of Na⁺ and Ca⁺ ions as compared to the control. The protein banding pattern showed an extra protein band in the presence of tannic acid as compared to the control culture. Tannase isolated from the bacterial isolates can find some applications in industry.

SURVEY OF PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF DRINKING WATER FROM VARIOUS AREAS OF LAHORE

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Water is vital to all life forms. There is no life without water. Human body is composed of two-third water. In order to maintain good health, proper proportion of drinking water is very important. Drinking water should be clear of any type of contamination. The biggest source of contamination in drinking water is pathogens. Water-borne pathogens results in water borne diseases. The purpose of this survey was to make finding of quality of drinking water in areas of central Lahore. It was observed that few areas including Choungi Amar Sadhu, Township and Greentown were highly contaminated with *T. coliforms*, *E. coli* and *Pseudomonas*. Although physical properties of water samples from these areas were satisfied showing no turbidity or odor. The chemical properties of all water samples were in accordance with the standards. The quality of drinking water should be checked in order to save the public from waterborne diseases.

ISOLATION AND MOLECULAR CHARACTERIZATION OF HEAVY METALS (CHROMIUM, COPPER, ZINC) RESISTANT BACTERIA FROM CONTAMINATED ENVIRONMENT AND ITS POTENTIAL ROLE IN BIOREMEDIATION

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Environmental pollution is a gift of industrialization. Industries discharge a huge amount of different heavy metals. Microorganisms found in these industrial effluents could be used to clean up the environments. In this research work, *Bacillus* species was isolated from water and soil samples that were collected from industrial effluents. It resisted Zn (5 mM), Cr (3 mM), Cu (2 mM), Pb (1 mM), Hg (1 mM) and Ni (0.5 mM). It showed an increased growth curve (OD₅₈₅ = 0.9 at 8th hour) after addition of 3 mM each metal (Zn, Cr, Cu) all together in a growth medium. Its growth was induced after 4 hours (OD₅₈₅ = 0.311) up to 7th hour (OD₅₈₅ = 0.723). The live cells could uptake more metals than dead cells. The metals uptake was observed more after 24 hours (48 %) up to 48 hours (70 %) and then decreased at 96 hour. Multiple metal resistance and uptake could make *Bacillus* species a potential candidate to be employed for bioremediation of industrial effluents.

CHARACTERIZATION OF ANTIBIOTIC RESISTANT GENE IN *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM LOWER RESPIRATORY TRACT INFECTION

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Streptococcus pneumoniae pose an emerging problem globally. This study was carried out to determine the prevalence of different pathogens in lower respiratory tract infections, and their antimicrobial susceptibility patterns. Sputum samples from each patient were collected aseptically, and inoculated on culture media. Out of a total of 250 samples 100 were positive cultures. Isolates were identified, and Antibiotic susceptibility patterns were determined using the Kirby-Bauer diffusion method. PCR assay for the detection of clinically relevant antibiotic resistance gene of *Streptococcus pneumoniae* was done. Conditions were optimized to determine antibiotic resistance pattern by PCR with primer designed for *ermB* (encoding macrolide resistance) gene in PCR amplification. The pathogens detected most frequently were *Streptococcus pneumoniae* 59 (59%) the next 18 (18%), isolates was of influenza virus followed by 13 (13%) isolates of *Staphylococcus aureus* and 10 (10%) mixed isolates of *Klebsiella* and *Pseudomonas* species respectively. The antibiotic susceptibility of most frequent pathogen *Streptococcus pneumoniae* was carried out and showed that 100 isolates of *S. pneumoniae* were resistant to erythromycin (45%), azithromycin (31.20%), clarithromycin (29.2%), ceftazidim (35.54%) and ciprofloxacin (21.59%) respectively while vancomycin has shown no resistance. Molecular methods hold the promise of an improved ability to rapidly detect microorganisms in respiratory secretions to make an accurate diagnosis and could be used in clinical as well as for epidemiologic necessities for accuracy in lower respiratory tract infections LRTI.

A NEW SPECIES OF GENUS *GANEO* KLEIN, 1905 COLLECTED FROM INDUS VALLEY BULLFROG *HOPLOBATRACHUS TIGERINUS* (ANURA: DICROGLOSSIDAE) OF DISTRICT UMERKOT, SINDH, PAKISTAN.

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A survey was conducted on the helminth parasites of Indus Valley Bullfrog *Hoplobatrachus tigerinus* of district Umerkot, Sindh, Pakistan. A total of 18 hosts were collected from different aquatic habitats and examined for the presence of helminth parasites. During examination of gut contents and visceral organs, three trematodes belonging to genus *Ganeo* Klein, 1905 were recovered from small intestine and identified as *Ganeo kabeeri* n.sp. Present species differs from its congeners on the basis of spinose body slightly tapering anteriorly; subterminal oral sucker larger than ventral sucker; shape of cirrus sac; shape and arrangement of testes; bilobed ovary attached with ventral sucker and posterior testis; distribution and extension of vitellaria; shape and extension of ceca and egg size. On the basis of these differentiating characteristics, a new species *Ganeo kabeeri* is proposed. The name of new species refers to Kabeer Ahmed, the father of first author.
