

PROCEEDINGS
OF
PAKISTAN CONGRESS OF ZOOLOGY

Volume 30, 2010

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



THIRTIETH PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

**THE ZOOLOGICAL SOCIETY OF PAKISTAN
AND
ISLAMIC AND EDUCATIONAL SCIENTIFIC & CULTURAL ORGANIZATION
(ISESCO) RABAT, MOROCCO**

at

UNIVERSITY OF AGRICULTURE, FAISALABAD

March 2 – 4, 2010

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Website: www.zsp.com.pk

ACKNOWLEDGMENTS

University of Agriculture, Faisalabad hosted the 30th Pakistan Congress of Zoology (International).

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

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30th PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

UNIVERSITY OF AGRICULTURE, FAISALABAD

March 2 – 4, 2010

PROGRAMME

TUESDAY, MARCH 2, 2010

08:30 AM	Registration
10:00 AM	Inauguration: Recitation from the Holy Quran
10:05 AM	Welcome Address by Prof. Dr. Muhammad Ashfaq, Dean, University of Agriculture, Faisalabad.
10:15 AM	Address by the President, Zoological Society of Pakistan
10:25 AM	Distribution of Medals and Awards
10:45 AM	Address by the Chief Guest Prof. Dr. Iqrar Ahmad Khan, Vice Chancellor, University of Agriculture, Faisalabad.
11:15 AM	Vote of Thanks by the Chairman, Department of Zoology, University of Agriculture, Faisalabad.
11:25 AM	Refreshment

JOINT SESSION I: (Plenary Lectures)

Chairperson: Prof. Dr. A.R. Shakoori

Co-chairperson: Prof. Dr. Imtiaz Ahmad

Speakers: 1. **Dr. Muhammad Perwaiz Iqbal**
Department of Biological and Biomedical Sciences, Aga Khan University, Karachi.

Hyperthromocysteinemia and β -vitamin deficiencies in a Pakistan Population in Karachi.

2. **Prof. Dr. Mohammad Anwar Waqar**

Distinguished National Professor, Dr. Panjwani Centre for Molecular Medicine and Drug Research University of Karachi, Karachi.

Asthma and allergies from fungal bio-aerosols in the atmosphere of Karachi

01:00 PM Lunch and Prayer

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

	Chairperson:	Prof. Dr. Anwar Waqar
	Co-chairperson:	Dr. Faiza Saleem
02:00 AM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. Perwaiz Iqbal
	Co-chairperson:	Dr. Bushra Muneer
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. Dil Ara Abbas Bukhari
06:45 AM	Paper reading	
08:00 PM	Dinner	

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

	Chairperson:	Prof. Dr. Shahnaz A. Rana
	Co-chairperson:	Dr. Jalal Arif
02:00 PM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. M. Suleman
	Co-chairperson:	Dr. Abida Butt
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. Mushtaq A. Saleem
	Co-chairperson:	Dr. Zulfiqar Ali Saqib
06:45 AM	Paper reading	
08:00 PM	Dinner	

HALL – 3

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

SESSION I

	Chairperson:	Prof. Dr. Mirza Azhar Baig
	Co-chairperson:	Dr. Uzma Khan
02:00 AM	Paper reading	
04:30 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. Ahmed Nadeem Sheri
	Co-chairperson:	Prof. Dr. Abdul Aleem Khan
05:00 PM	Paper reading	
06:30 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. Akbar Ali Khan
	Co-chairperson:	Dr. Muhammad Afzal
06:45 AM	Paper reading	
08:00 PM	Dinner	

WEDNESDAY, MARCH 3, 2010

JOINT SESSION II: (Plenary Lectures)

Chairman: Prof. Dr. M. Afzal Kazmi
Co-chairman: Prof. Dr. Fatima Mujeeb Bilqees

- 09:00 AM 1. **Prof. Dr. A.R. Shakoori**
Distinguished National Professor & Director, School of Biological Sciences, University of the Punjab, Lahore.
Role of Runx2 in epigenetic repression of ribosomal RNA gene.
2. **Prof. Dr. M. Suleman**
Department of Microbiology, Hazara University, Mansehra.
Recent outbreaks of Dengue in Northern Pakistan are due to reemergence of *Aedes aegypti*.
3. **Dr. Abdul Aleem Chaudhary**
Ex-DG Wildlife
Status of Wildlife and Wildlife Management in Pakistan

HALL – 1

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

SESSION IV

	Chairperson:	Prof. Dr. Javaid Iqbal Qazi
	Co-chairperson:	Dr. Abdul Rehman
10:00 AM	Paper reading	
11:00 PM	Tea Break	

SESSION V

	Chairperson:	Dr. M. Afzal Ghauri
	Co-chairperson:	Dr. Bushra Mirza
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION VI

	Chairperson:	Prof. Dr. Syed Shahid Ali
	Co-chairperson:	Dr. Farah R. Shakoori
02:00 PM	Paper reading	
04:30 PM	Tea Break	
05:00 PM	Executive Council Meeting	

SESSION VII

	Chairperson:	Dr. Shahid Nadeem
	Co-chairperson:	Mr. Ishtiaq Ahmad
05:00 PM	Paper reading	
06:30 PM	Prayer	
08:00 PM	Dinner	

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

	Chairperson:	Prof. Dr. Muhammad Ashfaq
	Co-chairperson:	Dr. Samina Qamar
10:00 AM	Paper reading	
11:00 PM	Tea Break	

SESSION II

	Chairperson:	Prof. Dr. Anjum Sohail
	Co-chairperson:	Dr. Ayesha Ihtesham
11:30 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION III

	Chairperson:	Prof. Dr. M.S. Wagan
	Co-chairperson:	Dr. Nasreen Muzaffar
02:00 PM	Paper reading	
04:30 PM	Tea Break	

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

SESSION VI

	Chairperson:	Prof. Dr. Q.B. Kazmi
	Co-chairperson:	Dr. Shahid Amjad
05:00 AM	Paper reading	
07:00 PM	Executive Council Meeting	
08:00 PM	Dinner	

HALL – 3

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

SESSION IV

	Chairperson:	Dr. Abdul Aleem Chaudhry
	Co-chairperson:	Dr. Mahmood ul Hassan
10:00 AM	Paper reading	
11:00 AM	Tea Break	

SESSION V

	Chairperson:	Prof. Dr. Shahid Mehboob Rana
	Co-chairperson:	Prof. Dr. N.T. Narejo
11:30 AM	Paper reading	
01:00 PM	Lunch Break and Prayer Break (Zuhar)	

SECTION IV: PARASITOLOGY

SESSION I

	Chairperson:	Prof. Dr. F.M. Bilqees
	Co-chairperson:	Prof. Dr. A.M. Dharejo
02:30 AM	Paper reading	
04:30 PM	Tea Break	

SESSION II

	Chairperson:	Dr. Nusrat Jahan
	Co-chairperson:	Dr. Asmatullah Kakar
05:00 PM	Paper reading	
06:30 PM	Prayer Break (Maghrib)	
07:00 PM	Executive Meeting	
08:00 PM	Dinner	

THURSDAY, MARCH 4, 2010

JOINT SESSION III: (Plenary Lectures)

Chairman: Prof. Dr. Nasim Siddiqi
Co-chairman: Prof. Dr. Shamsuddin Shaikh

- 09:00 AM 1. **Dr. Imtiaz Ahmad**
National Distinguished Professor, University of Karachi, Karachi.
Dengue outbreak in Karachi.
2. **Prof. Khalid Mahmood**
Department of Entomology, Faculty of Agriculture, Azad Jammu & Kashmir University, Rawlakot Campus, AK.
Agro-ecological basis for pest management.
3. **Prof. Dr. M. Nasim Siddiqi**
Former Chairman and Professor, Department of Zoology, University of Peshawar, Peshawar
Climate change, its effects, mitigations and adaptations

HALL – 1

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

SESSION VIII

Chairperson: Prof. Dr. Muhammad Ali
Co-chairperson: Prof. Dr. Naheed Ali

10:30 AM Paper reading
11:00 AM Tea Break

SESSION IX

Chairperson: Prof. Dr. Muhammad Shahab
Co-chairperson: Prof. Dr. Akram Shah
11:30 AM Paper reading
01:00 PM Lunch Break

SESSION X

Chairperson: Prof. Dr. Samina Jalali
Co-chairperson: Dr. Basharat Ahmad
02:00 AM Paper reading

HALL – 2

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

SESSION VII

Chairperson: Prof. Dr. Muhammad Akhtar
Co-chairperson: Dr. Abdul Aziz Khan
10:30 AM Paper reading
11:00 AM Tea Break

SECTION II: PEST AND PEST CONTROL

SESSION IV

Chairperson: Dr. Naheed Akhtar
Co-chairperson: Dr. Syeda Azra Qamar
11:30 AM Paper reading
01:00 PM Lunch Break (Zahur)

SESSION V

	Chairperson:	Prof. Dr. Shakila Mushtaq
	Co-chairperson:	Dr. Syeda Azra Tariq
02:00 PM	Paper reading	

SECTION IV: PARASITOLOGY**SESSION III**

	Chairperson:	Dr. Aly Khan
	Co-chairperson:	Prof. Dr. A.G. Arijo
10:30 AM	Paper reading	
11:00 AM	Tea Break	

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

	Chairperson:	Prof. Dr. Muhammad Javed
	Co-chairperson:	Dr. Zahida Tasawar
11:30 AM	Paper reading	
01:00 PM	Lunch Break (Zahur)	

SESSION IX

	Chairperson:	Prof. Dr. Afsar Mian
	Co-chairperson:	Prof. Dr. Iftikhar Ahmad
02:00 PM	Paper reading	
04:30 PM	Concluding Ceremony	
	Recitation	
	Congress Report by President ZSP	
	Award Ceremony	
	Concluding Remarks by the Chief Guest	
	Vote of Thanks	
05:30 PM	Refreshments	

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**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2010****Prof. Dr. A.R. Shakoori**

*Distinguish National Professor & Director, School of Biological Sciences,
University of the Punjab, Lahore.*

Professor Dr. Abdul Rauf Shakoori, Distinguished National Professor and Director, School of Biological Sciences, University of the Punjab is a well known Cell and Molecular Biologist, who has been actively involved in teaching and research for more than 48 years. Prof. Shakoori has a distinguished scientific career, which has previously been recognized by the Presidential Award of Aizaz-i-Kamal and a Civil Award Tamgha-i-Imtiaz. Prof. Shakoori is Fellow of prestigious Pakistan Academy of Sciences and the Academy of Sciences for the Developing World (TWAS). He has published more than 520 original research articles, produced 61 Ph.Ds. and written 17 books.

**RECIPIENT OF
LIFE TIME ACHIEVEMENT AWARD 2010**



Prof. Dr. Imtiaz Ahmad

Distinguish National Professor, Karachi University, Karachi

Prof. Imtiaz Ahmad obtained his Master's degree from University of Karachi in 1961 and Ph.D. from London University in 1963. He joined Zoology Department at Karachi University as Assistant Professor in 1965 and was appointed Chairman (1974-76, 1984-1987, 1987-88, 1994-97 and March through July 2000) and Professor in March, 1979. Since then Prof. Ahmad in his twenty monographs, 600 publications and half a dozen books designed pest forecasting models for rice and cotton insects and developed techniques of identification of areas of pest infestation for abandoning indiscriminate use of toxic chemicals. Under his guidance 17 students completed their Ph.D. and 85 their M.Phil/M.Sc. degrees by theses. Prof. Ahmad was awarded D.Sc. degree from London University in 1984, Senior Fulbright Fellowship in USA during 1985-1986, the award of the most outstanding research scholar of the Science Faculty, University of Karachi in 1968, 1985 and 1995, PARC-USDA Gold Medal in Plant Protection in 1985, Senior Scientist Gold Medal by the Pakistan Academy of Sciences in 1985, Gold Medal awarded by the Chancellor, University of Karachi in 1987, Golden Star and Cash Prize awarded by National Farm Guide Council/Hamdard Foundation in 1987,

Ambassadorship of Good-will awarded by Rotary International USA in 1988-89, Royal Society Fellowship at University of Wales, Cardiff, UK in 1992, in addition to several Gold Medals by Scientific and Cultural Society of USA/Canada, 1995, of PARC 1996, Biology Prize 1993 and Scientist of the year Award 1994 by National Book Foundation in 1997, Zoologist of the year Award 1996 by Zoological Society of Pakistan in 1997. He is Fellow of Pakistan Academy of Sciences, of National Environmental Sciences Academy of India, Honorary Fellow of Zoological Society of Bangladesh, Third World Academy of Sciences, Chief Editor of Journal of Sciences, University of Karachi, Journal of Entomology, Pakistan, Zoologica, Pakistan, Editor, Journal of Experimental Zoology of India, and served on editorial board of various national and international Journals i.e. Bulletin of Pure and Applied Sciences, India, and Oriental Insects, USA. He has been President of Entomological Society of Pakistan, has been Vice-President and President of Zoological Society of Pakistan. Dr. Ahmad, on August 14, 1998 was selected for Izaz-e-Kamal, the highest educational Government award, in January 1999 was awarded Chancellor's / Governor's Certificate of Appreciation and Presidential Award of Pride of Performance on 23 March 1999 for his outstanding research in Biology. He chaired National Environmental Sciences Academy of India Seminar in 1995 in New Delhi, and also Chaired a workshop on Phytopesticides in Montreal, Canada on the occasion of IUCN World Assembly. Last year the British Council awarded him a grant to attend 10th Auchenorrhyncha (leaf, tree and plant hoppers) Congress in Cardiff, U.K. (6th to 10th September) and through a Third World Academy of Sciences grant he attended their Workshop and Conference in Dakar, Senegal (20-26 November, 1999). He also visited University of Texas A&M, College Station, S.I.U, Carbondale and University of Connecticut, Storrs, USA (1996, 1997 & 1999) for research collaborations. Recently in October 2000 he was selected for invitational Lectures at Beltran University, Bogota, Colombia by TWAS, Trieste, Italy. Presently he is Meritorious Professor (in BPS-21). Acting Director Institute of Marine Sciences, of Biological Research Centre, Chairperson of Food Science and Technology Department and Dean Faculty of Science, University of Karachi.

**RECIPIENT OF
ZOOLOGIST OF THE YEAR AWARD 2010***



Prof. Dr. Muhammad Afzal

Professor, College of Agriculture, University of Sargodha

Dr. Muhammad Afzal, started his service career as Agricultural Officer in the Punjab Govt. in 1988 and then joined University of Agriculture, Faisalabad as Lecturer in 1990, where he served in the field of Agriculture Entomology for 21 years at different positions. In October 2008 he joined College of Agriculture, University of Sargodha as Professor of Entomology. He has published more than 130 research papers in journals of national and international repute. He has supervised 11 Ph.D. and 53 M.Sc. (Hons.) scholars. He also acted as Member Supervisory Committee for 86 M.Sc. (Hons.) scholars. He has completed three research projects as Principal Investigator. He is Life-Member of different national and international societies. Managing Editor of Monthly Magazine "Doab" and Chief Editor, International Journal of Agriculture and Applied Science (IJAAS).

In 2003, the Zoological Society of Pakistan honoured him with Prof. Mirza Azhar Beg Gold Medal for his significant contributions in the field of insect Ecology.

*Other nominee of this award was Dr. Muhammad Ather Rafi

**RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2010***



Dr. Bushra Mirza

Assistant Professor

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

Dr. Bushra Mirza obtained her M.Sc. and M.Phil degree (both with distinction) from Quaid-i-Azam University and then her Ph.D. degree from the University of Cambridge. During her Ph.D. she worked in the field of Plant Molecular Biology and while studying transgenic expression in *Arabidopsis*, she generated several transgenic lines. Some of these were later developed into the “the CAUT lines” for studies of cell autonomy in *Arabidopsis* and she also managed to clone a sequence used as marker (A32 on chromosome 1) in *Arabidopsis thaliana* R1 map (http://weedsworld.arabidopsis.org.uk/Vol3ii/mapping/All_chroms/html). After completing her Ph.D., she did a short post-doct involving cloning of a gene in *Arabidopsis* that affects the expression of transgenic. Soon after that she started teaching in Quaid-i-Azam University, first as visiting teacher (from 1997-1999) and then as regular faculty member (from 1999-todate). She has initiated projects involving not only diversity studies but also evaluation of medicinal activity of various plants and their genetic transformation for improvement. Seven Ph.D. and 57 M.Phil students have completed their research work successfully under her supervision. She has published about 50 papers in the Journals of national and international repute. She is also a co-author of book on bioethics published in Maryland, USA. As recognition of her research achievements, Pakistan Academy of Sciences has recently awarded her PAS Gold Medal (Biochemistry) of the year 2008.

*Other applicants for this award were Dr. Kausar Malik, Dr. Aqeel Javed, Dr. Habib-ur-Rehman and Mr. Muhammad Avais.

**RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2010***



Dr. Muhammad Sarwar

*Senior Scientist, Pakistan Atomic Energy Commission, Nuclear Institute of
Agriculture, Tandojam*

*Other applicant for this award was Dr. Abida Butt, Dr. Muhammad Hamid Bashir

**RECIPIENT OF
PROF. DR. NASIMA TIRMIZI GOLD MEDAL 2010***

Dr. Shahid Amjad

Ex-Dean, Faculty of Marine Sciences, University of Lasbela, Balochistan

Prof. Dr. Shahid Amjad is a Ph.D. in Biological Oceanography from University of Wales (UK). He was Director General, National Institute of Oceanography, Karachi for 8 years from 1996 to 2004.

In 2005 Dr. Amjad established the Marine Sciences Faculty at Lasbela University of Balochistan and served as Founding Dean in the Faculty of Marine Sciences for over 3 years. Dr. Amjad has supervised Ph.D./M.Phil students at the University of Karachi. He has over 30 research publications to his credit, published in journals of national and international repute. He is a visiting faculty member at University of Karachi and the NED Engineering University where he teaches courses on Environment and Coastal Management. He has been engaged in marine research and management of marine related issues for over 24 years.

*Other applicant for this award was Prof. Dr. Naureen Aziz Qureshi,

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

1. Muzaffar Ahmad Gold Medal 2010

Fourteenth Muzaffar Ahmad Gold Medal 2010 was received by Ms. Mehwish Riaz for obtaining first position in the M.Sc. Zoology examination of the University of the Punjab.



Mehwish Riaz

2. Ahmed Mohiuddin Memorial Gold Medal 2010

Seventh Ahmed Mohiuddin Memorial Gold Medal 2010 was given to Ms. Sobia Aziz, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

3. Muhammad Afzal Hussain Memorial Gold 2010

Twelfth Muhammad Afzal Hussain Memorial Gold 2010 was given to Ms. Nazia Arshad for obtaining first position in Parasitology for her M.Sc. Zoology examination of the University of Karachi.

4. Mujib Memorial Gold Medal 2010

Fourteenth Mujib Memorial Gold Medal 2010 was given to Ms. Hira Shaikh who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

5. Prof. Imtiaz Ahmad Gold Medal 2010

Seven Prof. Imtiaz Ahmad Gold Medal 2010 was given to Ms. Nimra Qureshi who obtained first position in the M.Sc. Zoology examination in Entomology, University of Karachi, Karachi.

5. Afsar Mian Gold Medal 2010

First Afsar Mian Gold Medal 2010 was given to Ms. Madiha Zahid who obtained first position in the M.Sc. Biology/Zoology examination of the Arid Agriculture University, Rawalpindi.



Madiha Zahid

6. Ahmed Mohiuddin Memorial Gold Medal 2010

Nine Ahmed Mohiuddin Memorial Gold Medal 2010 was given to Mr. Sobia Aziz, who obtained first position in the M.Sc. Zoology examination of the University of Sindh, Jamshoro.

7. The M.A.H. Qadri Memorial Gold Medal 2010

Eleventh M.A.H. Qadri Memorial Gold Medal 2010 was given to Dr. Shamim Fatima for his Ph.D. degree in Zoology specializing in the field of Parasitology from University of Karachi.

8. Prof. Dr. S.N.H. Naqvi Gold Medal 2010

Six Prof. Dr. S.N.H. Naqvi Gold Medal 2010 was given to Dr. Tahir Ali for his Ph.D. degree in Zoology specializing in the field of Toxicology from University of Karachi, Karachi.

FISHES OF THE RIVER ATTOCK GORGE WITH NEW RECORD OF *BATASIO PAKISTANICUS*

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Abstract.- River Indus takes its origin in the south western Tibet. It enters in district Attock at Momanpura. It contracts in to a narrow rocky bed after joining of Kabul River on its right bank. It rushes on through a gorge with a high bank on each side, having Attock Fort on its left bank. Below Attock Khurd near Bagh Nilab it again spreads out into a kind of lake. But soon again contracts and flows through a gorge down to Makhad and hence out beyond the district boundaries after flowing about 95 Km. Total 31 number of fish species belonging to 28 genera 12 families and 6 orders have been collected with a new record of *Batasio pakistanicus* (Family Bagridae). Previously, this species was described from Jinnah Barrage in the Mianwali District. Its new locality is situated about 150 km upstream.

Key words: Fishes, Attock Gorge, *Batasio pakistanicus*.

INTRODUCTION

The River Indus originates in the South Western Tibet. It is largely derived from snow water and subject to the tremendous floods. It flows in the north-west between the Karakoram and the Himalayas. In the North West Frontier Province (NWFP) it falls into the Tarbela Lake. After covering about 50 km it receives the River Kabul on its right bank at Attock Khurd and flows through a gorge with a high banks on each side, having Attock Fort on its left bank. Below Attock Khurd, near Bagh Nilab, it again spreads out into a kind of lake, but soon it contracts and flows through narrow gorges down to Makhad and hence out beyond the district boundaries after covering about 95 km. It flows through the pothohar plateau and the salt Range and then enters the Indus Plain in the south of Kalabagh (Attock District Gazetteer, 1930).

MATERIALS AND METHODS

The present studies were carried out during 2004 to 2007 at three localities Attock Khurd (confluence of River Indus & Kabul), Brotha confluence of river

Indus & Haro), Garyana river Haro in six visits two at each locality. The collection of fish was made by cast nets, drag nets and scoop nets. The specimens were preserved in 10% solution of Formalin and then shifted in 70% alcohol. The specimens were identified following Mirza M.R and Sandhu A.A 2007.

RESULTS

Total 31 fish species were identified with a new record of *Batasio Pakistanicus* belonging to 28 genera, 9 families and 6 orders.

SYSTEMATIC ACCOUNT

All fishes belong to class Actinopterygii, subclass Neopterygii and infraclass Teleostei.

COHORT EUTELEOSTEI
SUPERORDER OSTARIOPHYSI
ORDER CYPRINIFORMES
FAMILY CYPRINIDAE
Subfamily Cultrinae
Genus *Chela* Hamilton

1. *Chela cachi* (Hamilton)

A small sized fish. One specimen 5cm in total length was caught from Attock Khurd.

Genus *Salmophasia* Swainson

2. *Salmophasia bacaila* (Hamilton)

There are 3 specimens of this species caught from Attock Khurd with maximum length of 13cm.

Subfamily Aspidoparinae
Genus *Aspidoparia* Heckel

3. *Aspidoparia morar* (Hamilton)

Moderate sized fish. Total 6 specimens were collected from Attock Khurd and Brotha localities with maximum length of 10.9cm.

Subfamily Rasborinae
Genus *Barilius* Hamilton

4. *Barilius modestus* (Day)

A small sized fish with two pairs of barbels. Only one specimen was caught from Garyala river Haro.

5. *Barilius pakistanicus* Mirza & Sadiq

Body cylindrical , with vertical bars on the lateral sides. There are 2 specimens of this species caught from Garyala river Hero with maximum length of 6.5cm.

Genus *Devario* Heckel

6. *Devario devario* (Hamilton)

A fish with laterally compressed body, mouth, superior and without barbels. There are 5 specimens of this species collected from Brotha and Garyala with maximum length of 10cm.

Subfamily Barbinae
Genus *Cyprinion* Heckel

7. *Cyprinion watsoni* (Day)

There are 15 specimens of this fish collected from Garyala river Haro ranging from 6.7 to 10.5 cm in total length.

Genus *Labeo* Cuvier

8. *Labeo diplostomus* (Heckel)

A fish with subcylindrical body, head short, mouth sub-terminal, maxillary barbels very short. There are 4 specimens of this species ranging from 17.5 to 18.8cm in total length.

Genus *Puntius* Hamilton9. *Puntius chola* (Hamilton)

A small sized fish with two barbels and a dark blotch on the side of caudal peduncle. There are 6 specimens in the collection from Garyala having maximum length of 9.5 cm.

10. *Puntius conchonius* (Hamilton)

A small sized fish, mouth anterior and without barbels. There are 8 specimens from Garyala with maximum length of 7cm.

11. *Puntius sophore* (Hamilton)

Fish with elongated body, head short, eyes large, mouth small and terminal, without barbels. There are 47 specimens in the collection with maximum length of 8.5 cm.

Genus *Tor* Gray12. *Tor macrolepis* (Heckel)

Body elongated, perfectly streamlined, mouth sub-terminal. There are 8 specimens in collection from Attock Khurd, Brotha and Garyala ranging from 16.3 to 19.2cm.

Subfamily Garrinae

Genus *Crossocheilus* Hasselt13. *Crossocheilus diplocheilus* (Heckel)

A medium sized fish, dorsal profile arched, two pairs of very small rostral and maxillary barbels. There are 14 specimens in collection with maximum length of 13.8 cm from three localities.

Subfamily Schizothoracinae

Genus *Racoma* McClelland14. *Racoma labiata* McClelland & Griffith

A medium sized fish, mouth ventral with two pairs of barbels. Lower lip

modified into a complete labial fold. There are two specimens in the collection from Brotha and Garyala with maximum length of 12.8cm.

Grnus *Schizothorax* Heckel

15. *Schizothorax plagiostomus* Heckel

A medium sized fish with sub-cylindrical body, head short, triangular, mouth ventral with two pairs of barbels. There are 8 specimens in the collection ranging from 13.8 to 18 cm in total length caught from all localities.

Genus *Carassius* Nilsson

16. *Carassius auratus* (Linnaeus)

A fish with muscular and deep body, mouth small and without barbels. There are 3 specimens in collection from Attock Khurd with maximum length of 16.7cm.

Genus *Cyprinus* Linnaeus

17. *Cyprinus carpio* Linnaeus

Body strongly developed, mouth with two pairs of barbels, maxillary larger than rostral. There is one specimen in collection caught from Attock Khurd with total length of 11.5cm.

FAMILY NEMACHEILIDAE

Genus *Acanthocobitis* Peters

18. *Acanthocobitis botia* (Hamilton)

A small sized fish. Only one specimen was collected from Garyala with total length 4.5cm.

ORDER SILURIFORMES

FAMILY BAGRIDAE

Subfamily Bagrinae

Genus *Batasio* Blyth19. *Batasio pakistanicus* Mirza & Jan

A small sized fish with a dark blotch on occipit , a faint streak along the lateral line from shoulder spot to end of caudal peduncle forming a faint spot. Adipose dorsal slightly dark in the middle of its base. Caudal black tipped. One specimen was collected from Attock Khurd with total length 6.5cm and standard length 5cm.

Genus *Mystus* Scopoli20. *Mystus bleekeri* (Day)

Fish with stout, muscular body, occipital process long, barbels eight: maxillary barbels not extending beyond the anal fin. There are 6 specimens collected from Attock Khurd and Garyala ranging from 10.8 to 12.4 cm in total length.

FAMILY SILURIDAE

Genus *Ompok* Lacepede21. *Ompok pabda* (Hamilton)

Body laterally compressed, maxillary barbels reaching the pectoral fin. One specimen was collected from Brotha with total length of 16.3cm.

FAMILY SCHILBEIDAE

Subfamily schilbeinae

Genus *Clupisoma* Swainson22. *Clupisoma naziri* Mirza & Awan

Body compressed, head short, dorsal with a spine, anal fin with more than

40 rays, adipose dorsal present. There are 3 specimens caught from Attock Khurd with maximum length of 15 cm.

FAMILY SISORIDAE

Genus *Gagata* Bleeker

23. *Gagata cenia* (Hamilton)

A small sized fish with eight barbels, maxillary barbels not reaching the base of the pelvic fins. There are 8 specimens in collection with maximum length of 6.8cm from Attock Khurd and Brotha.

Genus *Glyptothorax* Blyth

24. *Glyptothorax punjabensis* Mirza & Kashmiri

A fish with dorsal spine strong and serrated. There are 14 specimens caught from Bortha and Garyala ranging from 14.8 to 15.5 cm in total length.

FAMILY HETEROPNEUSTIDAE

Genus *Heteropneustes* Muller

25. *Heteropneustes fossilis* (Bloch)

A médium sized fish ,pectoral fin strong and serrated. There is one specimen with total length 17.2cm caught from Garyala.

SUPERORDER ACANTHOPTERYGII

SERIES ATHERINOMORPHA

ORDER BELONIFORMES

FAMILY BELONIDAE

Genus *Xenentodon* Regan

26. *Xenentodon cancila* (Hamilton)

A fish with cylindrical body, jaws form a long beak. There are 4 specimens caught from Attock Khurd and Garyala with maximum length of 30.5 cm.

SERIES PERCOMORPHA
ORDER CHANNIFORMES
FAMILY CHANNIDAE
Genus *Channa* Scopoli

27. *Channa punctata* (Bloch)

Body elongated, pelvic fin more than half of the length of pectoral fin. There are 2 specimens in collection with maximum length of 10.2cm from Garyala.

ORDER MASTACEMBELIFORMES
FAMILY MASTACEMBELIDAE
Genus *Mastacembelus* Scopoli

28. *Mastacembelus armatus* (Lacepede)

Body eel shaped, dorsal fin long with sharp pointed spines. There are 13 specimens of this species ranging from 22 to 32 cm in total length from Brotha and Garyala.

ORDER PERCIFORMES
FAMILY CHANDIDAE
Genus *Chanda* Hamilton

29. *Chanda nama* Hamilton

A small fish with transparent body, major internal organs visible. There are 3 specimens caught from Attock Khurd and Garyala with maximum length of 7cm.

Genus *Parambassis* Bleeker

30. *Parambassis ranga* (Hamilton)

Body deep and compressed, mouth oblique. Only one specimen was caught from Attock Khurd having 7cm length.

FAMILY CICHLIDAE
Genus *Oreochromis* Gunther

31. *Oreochromis aureus* (Steindachner)

Body with vertical bars. There are 34 specimens collected from three localities with maximum length of 14.7 cm. It is in abundance and widely distributed.

DISCUSSION

The part of river Indus under study contains a large number of South Asian and a few exotic fish species. Only 31 species belonging to 28 genera, 12 families and 6 orders have been reported. Among them *Cyprinion watsoni*, *Puntius sophore*, *Crossocheilus diplocheilus*, *Glyptothorax punjabensis*, *Mastacembelus armatus* and *Oreochromis aureus* were in abundance, *Puntius conchoni*, *Schizothorax plagiostomus* and *Gagata cenia* were very common, followed by *Salmophasia bacaila*, *Aspidoparia morar*, *Devatio devatio*, *Labeo diplostomus*, *Puntius chola*, *Tor macrolepis*, *Carrassius auratus*, *Mystus bleekeri*, *Clupisoma naziri*, *Xenentodon cancila* and *Chanda nama* were not common.

Among these *Chela cachius*, *Barilius modestus*, *Barilus pakistanicus*, *Racoma labiata*, *Cyprinus carpio*, *Acanthocobitis botia*, *Batasio pakistanicus*, *Ompok pabda*, *Heteropneustes fossilis*, *Channa punctata* and *Parambassis ranga*, were rare. *Batasio pakistanicus* is a new record from Attock Khurd as it has been collected previously from Jinnah Barrage District Mianwali. Now its new locality is situated about 150Km upstream. Some more species recorded by Javed *et al.* (2005) from Attock Khurd and Garyala but absent in this report include *Notopterus notopterus*, *Amblypharyngodon mola*, *Systomus sarana*, *Gibelion catla*, *Labeo calbasu*, *Labeo dyocheilus pakistanicus*, *Puntius ticto*, *Rita rita*, *Channa striata*, *Glossogobius giuris* and *Colisa fasciata*. Moreover, Ali *et al.* (1980) has reported following species *Ompok bimaculatus* and *Nemacheilus corica* from Attock Khurd but has not been caught in present collection.

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NUTRITIONAL IMPROVEMENT IN A BACTERIALLY SOLID STATE FERMENTED FORMULATED FISH FEED

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Abstract.- A formulated fish feed was fermented employing solid state fermentation, a well known process for increasing nutritional status of feed and other products. *Bacillus cereus* fermentation caused 17% and about 16% increases in protein and carbohydrate contents, respectively of the feed within 24 hrs. While the bacterium *Sporilactobacillus inulinis* could cause 50% and 58% increases in the protein and carbohydrate contents, respectively of the 24 hrs fermented feed. At the end of fermentation period i.e. 168 hrs, the feed fermented by *Edwardsiella hoshinae* had 45, 180 and 165% higher than the respective control values of protein, carbohydrate and lipid contents, respectively. About 6, 100 and 89% elevations in these parameters were recorded for the feed fermented by employing a strain of *Arthrobacter* sp. The bacteria reported in this communication increased the three major categories of nutrients upto several folds as compared to the non-fermented (control) feed. Provision of such fermented feed is expected to increase the fish yield and promote aquaculture in this country. While taking into account differential potential of the bacterial isolates in terms of increasing soluble protein, carbohydrate and fat contents, respective microbial species can be employed to upgrade nutrient(s) required specifically for a particular available feed.

Key words: Protein, Food , Carbohydrate, Feed lipids, Fermented Feed.

INTRODUCTION

Success in aquaculture is based on various criteria, in which selection of suitable feed and its optimum use is very important. Growth of fish at all stages is largely governed by the kind of food, feeding frequency, food intake and absorption of the nutrients. Proteins and lipids are major components of formulated fish feed. Fish have higher dietary protein requirement. When culturing carnivorous fish, protein usually accounts for 40-50% of feed dry matter, most of which is supplied by fish meal because of its nutritional value and palatability (Wilson and Halver, 1986; Hardy, 1999). Dietary lipids play a major role in providing a source of concentrated energy and essential fatty acids, especially for carnivorous fish as these species have a limited ability to utilize

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carbohydrates as an energy source (Oliva-Teles, 2000). Several studies have shown that providing adequate energy with dietary lipids can minimize the use of more higher priced protein as an energy source (Al *et al.*, 2004; Hung *et al.*, 2004; Kim and Lee, 2005). Beside trials of different recipes of fish feeds, microbial fermentations have been considered to improve given feeds' nutritional aspects (Pandey, 2003).

Rohu is a popular fish in Pakistan. It is cultured on large scale by private sector. Rohu fingerlings showed better growth and feed utilization efficiency when fed with diets containing fermented seed meal than those fed with diet containing raw seed meal (Ramachandran *et al.*, 2004). The present study was aimed at fermenting a formulated fish feed by employing solid state fermentation (SSF). The SSF was conducted with pure cultures of four bacterial isolates obtained from culture collection of this microbiology laboratory of department of Zoology, University of the Punjab, Lahore. The fermented feed had several fold increases in carbohydrate, lipid and protein contents of the feed.

MATERIALS AND METHODS

Morphological and physiochemical characterization of the bacteria:

The bacterial isolates were obtained from our stock culture collection. Fresh cultures of the bacteria were processed for Gram's and endospore stainings, motility, oxidase, catalase, and Voges-Proskauer tests as described by Benson (1994). While flagellar staining and lecithinase and nitrate reduction tests were performed after Collins *et al.* (1985). The Citrate utilization test was performed as mentioned in Merck, (1996-1997). Based upon the characteristics determined the bacterial isolates were identified as described by Holt *et al.* (2000).

Growth conditions' optimization

The bacteria were optimized for temperature, pH, aeration and inocula size, while culturing in nutrient broth. A single bacterial colony was inoculated into 5 ml of sterilized nutrient broth and the test tubes were incubated at 28°C, 37°C and 50°C for 18 hrs. The growth was then determined by measuring O.D. at 600 nm. For pH optimization the bacteria were inoculated in broths having initial pH values of 5, 7 and 9 and incubated at their respective optimum temperatures. The bacteria were inoculated into the broths of corresponding optimum pH and incubated for 18 hrs at their optimum temperatures on orbital shaker at 100 rpm

and without aeration. Then each bacterial strain was inoculated in sterilized nutrient broth with optimum pH representing 1, 5 and 10% inocula and incubated at their corresponding optimum temperatures and oxygen requirements for 18 hrs. Growth of all the cultures was then determined as described before.

Experimental procedure

Solid state fermentation of fish feed

Fish feed; a mixture of (%) fish meal (5), rice polish (34.3), ground nut oil cake (53.7), molasses (4.0), dicalcium phosphate (1.0), table salt (1.0) and vitamin premix (1.0) was used in this study. Bacterial inocula were prepared by centrifuging overnight incubated bacterial cultures in nutrient broth at 5,000 rpm for 10 min at 4°C and then suspending the pellet in sterile 0.89% saline solution. This process was repeated once again, and the cell concentration in the final suspension within the saline was adjusted to an optical density of 0.5 at 600nm.

10 gm of the fish feed was autoclaved at 121°C for half an hour. Then autoclaved cooled water containing 10% of a given bacterial inoculum was added to represent 70% water content of the inoculated feed. Each bottle was fitted with an inlet pipe to provide filtered aeration with the help of an electric aerator and outlet cotton plugged pipe to exhaust the spent air. Samples were taken after 24, 48, 72, 120 and 168 hrs under sterilized conditions. Weighed amounts of the samples were dried at 105°C till the constant weight to determine their moisture contents. Amount of autoclaved water was added daily to compensate its loss based upon previous samples' moisture contents.

Biochemical analysis of the feed

Half gram of a given fish feed sample was homogenized in 6 ml of 0.89% saline. The material was centrifuged at 5000 rpm for 10 minutes. Supernatant was proceeded for the estimation of total carbohydrates and proteins by the methods of Dubois *et al.* (1956) and Lowry *et al.* (1951), respectively. For lipids determination, 0.5 gm of a sample was suspended in 3 ml of 95.5% ethyl alcohol and the test tubes were covered and kept in water bath for 15 minutes for boiling the contents. Volume within each test tube was then regained up to 3 ml with the addition of ethanol. The test tubes were kept at 37°C for 24 hrs and then centrifuged at 5000 rpm for 10 minutes. The supernatant was proceeded for the estimation of lipids according to Zollner and Kirsch (1962). Statistical analysis was performed according to SPSS12 program. For comparison of two and more

than two groups, student-t-test and single factor analysis of variance were applied, respectively.

RESULTS AND DISCUSSION

Four bacterial isolates obtained from stock culture of the laboratory were further characterized and optimized for their growth conditions in nutrient broth. After 18 hrs of incubation at different temperatures the bacterial isolates designated as SM1, A4 and P16 showed best growth at 37°C, while L7 grew best at 50°C (Fig. 1). The SM1, A4, L7 and P16 showed best growth with initial pH 7. Under respective optimum conditions of temperature and pH, the P16 and L7 bacteria revealed highest growth with 5% inoculum, while A4 and SM1 grew well with 1% and 10% inocula respectively (Fig.1). Under the respective optimum conditions of temperature and pH, the bacterial isolates A4, L7 and SM1 showed highest growth when they were provided aeration on orbital shaker at 100 rpm. While the strain P16 showed highest growth without aeration (Fig.1).

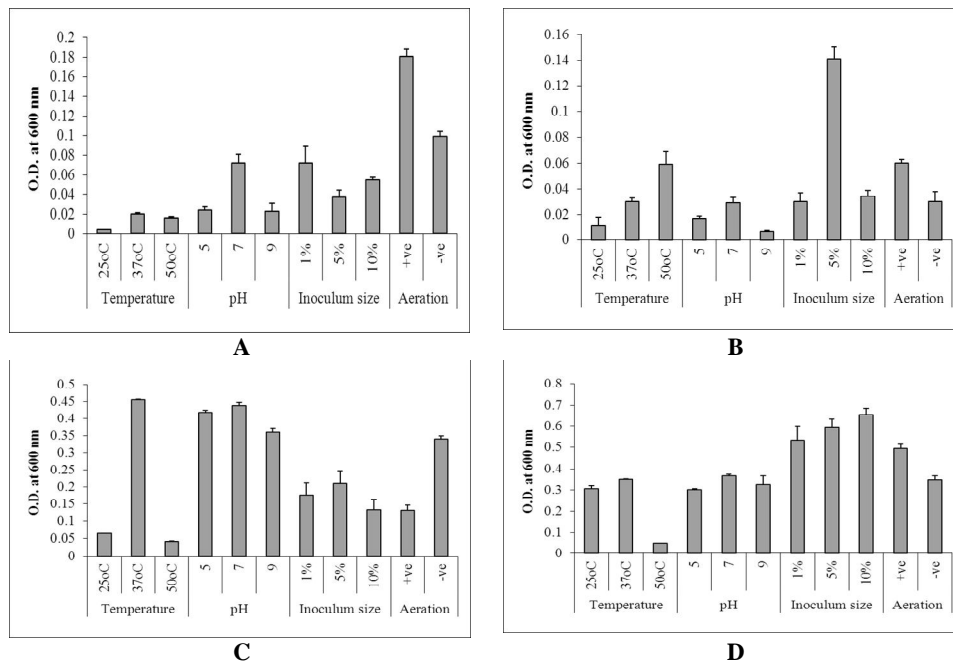


Fig. 1. Growth conditions optimizations of the bacterial species *B. cereus* (A), *Sporilactobacillus inilunis* (B), *E. hoshinae* (C) and *Arthrobacter* sp. (D).

Characteristics and identification of the bacteria

Various morphological and physicochemical characteristics of the bacterial isolates are shown in Table I. Based upon these characteristics, the bacteria were identified after Holt *et al.* (2000). Accordingly the isolates designated as L7, A4, P16 and SM1 hereafter will be described as *Sporolactobacillus inilunis*, *Bacillus cereus*, *Edwardsiella hoshina* and *Arthrobacter* sp. respectively.

Solid state fermentation of the feed

Treatment of the feed with *Bacillus cereus* caused 17% increase in protein content after 24 hrs which enhanced significantly upto 120% at 168 hrs of fermentation (Fig. 2A). Protein content of the control (uninoculated) feed ranged from 9.43 ± 0.07 to 10.10 ± 0.46 mg/gm through the study period. Carbohydrate content of the control feed ranged from $25.18 \pm$ to 26.94 ± 0.58 mg/gm. While that of the fermented feed the parameter showed 15.8 and 8.46% increases at first and last sampling periods, respectively. For the remaining study points, the carbohydrates decreased compared to the respective control values. Lipid content of the control feed fluctuated from 371.81 ± 31.62 to 395.9 ± 21.69 mg/gm. The fermented feed showed decreases upto first three points followed by 52.91 and 33.7% elevations in the parameter at second last and the last study periods, respectively (Fig. 2A). Along with the same batch of the control another experiment yielded that *Sporilactobacillus inilunis* fermented feed, could attain about 50% increase in the protein contents right at the first sampling point. For the remaining study periods comparable elevations were not observed. Likewise, carbohydrates increased upto 58% after 24 hrs of fermentation followed by a lesser increase and then decreases as compared to the respective control values. Lipids content of the fermented feed decreased throughout the study periods, with deficits raising from 0.12 to 21.14% at different points, except for the last study period for which about 13% elevation over the control value was observed (Fig. 2B).

In second batch of the experiment, wherein the control feed during the fermentation period was found to contain, 10.19 ± 0.20 to 12.26 ± 1.20 , 45.05 ± 0.65 to 45.83 ± 0.39 and 150.90 ± 11.04 to 158.11 ± 21.95 mg/gm of protein, carbohydrate and lipid contents, respectively. The feed fermented with *Edwardsiella hoshinae* and *Arthrobacter* sp. had varying levels of nutritional improvements. In case of *E. hoshinae* fermented feed, protein, carbohydrate and lipids increased upto 50.4, 62.8 and 16.62%, respectively over the respective

TABLE I.- MORPHOLOGICAL AND PHYSIOCHEMICAL CHARACTERISTICS OF THE BACTERIAL ISOLATES AND THEIR IDENTIFICATION.

Characteristic	Bacterial Isolates' code			
	A4	L7	P16	SM1
Colony Morphology	Creamy, white in middle, round with radiating entire margins with umbonate elevation opaque and viscous.	White, irregular and spreading with lobate margins, flat elevation and opaque.	Yellow irregular with spreading and branching margins, flat elevation and opaque.	Creamy, round with entire margins, flat elevation and viscous.
Gram's Reaction	+ve Bacilli	+ve Bacilli	-ve Bacilli	+ve Bacilli
Endospore	+ve	+ve	-ve	-ve
Flagella	-ve	-ve	+ve polar tuft	---
Motility Test	+ve	+ve	+ve	-ve
Oxidase Test	+ve	+ve	-ve	-ve
Catalase Test	+ve	+ve	+ve	+ve
Nitrate Reduction test	+ve	-ve	+ve	---
Voges-proskauer Test	+ve	+ve	-ve	+ve
Methyl Red Test	+ve	-ve	+ve	-ve
Lecithinase Test	+ve	---	---	---
Simmon' citrate Test	-ve	-ve	-ve	---
Growth on MacConkey agar	-ve	-ve	+ve	---
Glucose Oxidation	---	---	+ve	---
Fermentation	-ve	+ve	-ve	---
H ₂ S Production				
Identification	<i>Bacillus cereus</i>	<i>Sporolactobacillus inulinis</i>	<i>Edwardsiella hoshinae</i>	<i>Arthrobacter sp.</i>

--- = Test was not required.

control values at 24 hrs stage. While at end of the fermentation these parameters showed about 45, 180 and 165% increases over the respective control values (Fig. 2C). *Arthrobacter* sp. could cause elevations in the protein, carbohydrate and lipid contents of the 168 hrs fermented feed upto 6.41, 100.3 and 89.2%, respectively (Fig. 2D).

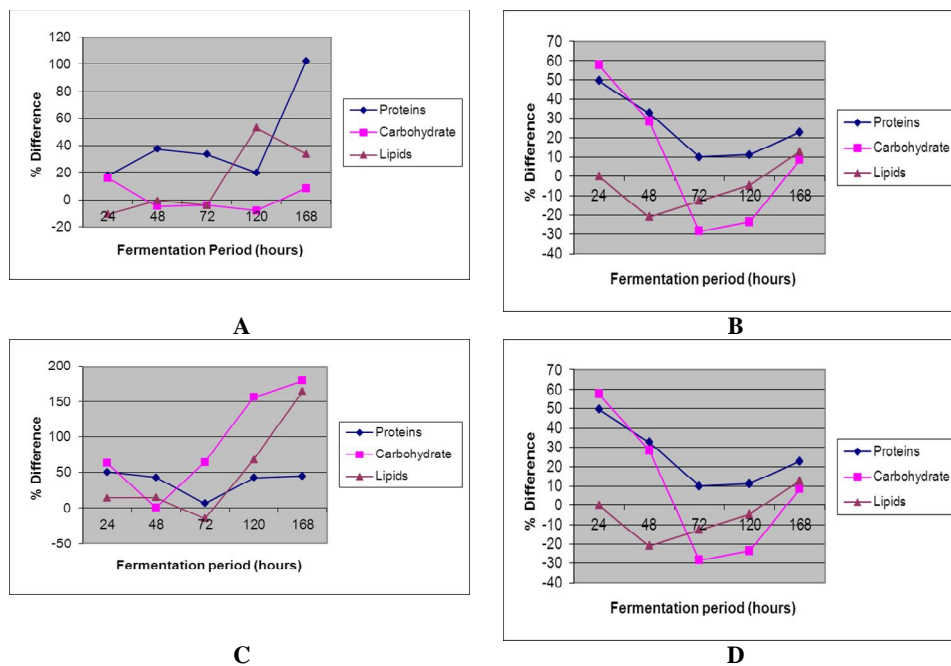


Fig. 2. % changes in the protein, total carbohydrates and lipids contents (mg/gm) of the fish feed subjected to solid state fermentation employing *B. cereus* (A), *Sporilactobacillus inulinus* (B), *E. hoshinae* (C) and *Arthrobacter* sp. (D)

Solid state cultivation of useful microorganisms is one of the preferred methods for nutritional enrichment of agricultural surplus, residues and other products. Objective of this study was to determine effects on certain nutritional parameters of a formulated fish feed following SSF. Bacterial growth on fish feed caused significant increases in protein, carbohydrate and lipid contents. Effects of dietary protein and lipid levels on growth performance of fishes are well documented (Hamre *et al.*, 2004; Kim and Lee, 2005). The interesting observation was that the four bacterial species employed in this study did not only vary in terms of degrees of elevations in the nutritional parameters of the SSF feed, but they also needed different incubation periods for yielding

maximum increase in a given parameter. For instance, the *B. cereus* showed over 100% increase in the protein content of fermented feed at the end of fermentation period, while the bacteria *S. inilunis* and *E. hoshinae* caused maximum but upto 50% increase in the protein content after 24 hrs of the fermentation. Still another category of the differences appeared in terms of kind of the nutritional parameter that showed maximum increase. For example, *B. cereus* yielded maximum increase in protein content (about 100%) at end of the observation period. While the bacteria, *S. inilunis* and *E. hoshinae* yielded maximum elevations in carbohydrate contents of SSF feed upto about 58 and 180% at first and last study periods, respectively. The latter bacterium yielded a maximum increase of about 165% in lipid content after 168 hrs against all the fermentations carried out in this study. In fact adequate lipid level in diet is important for the growth of fish and product quality (Hamre *et al.*, 2004; Lopez *et al.*, 2005). Differences in terms of maximum increase of specific nutritional parameter(s) and specific time frame required for bringing elevation(s) in them are highly valuable in designing SSF processes for nutritionally upgrading fish feeds of varying nature. For instance, if the original feed is deficient in lipid contents then the bacteria which specifically may enhance this parameter might be employed. Likewise, the fermentation agents which brought increases in protein content earlier may be used to reduce cost of the fermentation process. These information allow selective use of the bacterial isolates for enhancing specific nutritional parameters in the fish feed. Inasmuch the fish promoting effects of the fermented feed are concerned, the biochemical evaluations of the fermented feeds are promising. However, their biological assays are required before practical application of the bacterially fermented fish feed is considered.

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Flagella	-ve	-ve	+ve polar tuft	---
Motility Test	+ve	+ve	+ve	-ve
Oxidase Test	+ve	+ve	-ve	-ve
Catalase Test	+ve	+ve	+ve	+ve
Nitrate Reduction test	+ve	-ve	+ve	---
Voges-proskauer Test	+ve	+ve	-ve	+ve
Methyl Red Test	+ve	-ve	+ve	-ve
Lecithinase Test	+ve	---	---	---
Simmon' citrate Test	-ve	-ve	-ve	---
Growth on MacConkey agar	-ve	-ve	+ve	---
Glucose Oxidation Fermentation	---	---	+ve	---
H ₂ S Production	-ve	+ve	-ve	---
Identification	<i>Bacillus cereus</i>	<i>Sporolactobacillus inilunis</i>	<i>Edwardsiella hoshinae</i>	<i>Arthrobacter sp.</i>

--- = Test was not required.

ISOLATION AND SCREENING OF CHITINOLYTIC BACTERIA FROM SOIL SAMPLES

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Abstract.- Chitin, a major constituent of insects and other arthropods' exoskeleton, could be hydrolyzed by chitinases which in turn have many applications and potential uses. Culturable chitinolytic bacterial diversity was collected from insects' influenced soils. Colloidal chitin was prepared by treating commercially available crude chitin with hydrochloric acid and ethanol. Chitinase production was noticeably influenced by the cultural medium. A total of 25 chitinolytic isolates were isolated among which five showed prominent zones of clearance when cultivated on colloidal chitin agar plates or three days colonial and cellular morphology of all the isolates are reported here with a note on their chitinolytic and application potentials.

Key words: Chitin, Bacterial chitinases, Biological control.

INTRODUCTION

Chitin is insoluble linear β -1,4- linked polymer of N- acetylglucosamine. It is abundant renewable natural source biologically pooled in mollusks, arthropods, fungi and algae inhabitant of both freshwater and marine environments in addition to soil (Nicol, 1991; Gooday, 1990; Sheikh and Deshpande, 1993; Wang and Chang, 1997). The peritrophic membrane and exoskeleton of insects act as physicochemical barriers to environmental hazards and predators. Both are composite materials made up primarily of chitin and protein, with the latter also containing some lipids, catecholamine metabolites, minerals, and other minor components (Kramer and Muthukrishnan, 1997). About 10% of the global landing of aquatic products consists of organisms rich in chitinous material. These include shrimps, crabs, squids, oyster, krill and cuttlefish. More than 80,000 metric tons of chitin is obtained per year from marine wastes (Subasinghe, 1995; Wang and Chang, 1997). Dynamic processes such as molting of cuticles as well as senescence results in a continuous rain of chitin to the ocean floor known as marine snow (Alldredge and Gotschalk, 1990; Wang and Chang, 1997; Cohen-Kupiec and Chet, 1998). While there is no substantial accumulation of chitin in the ocean sediments, as it is effectively degraded and catabolised by bacteria (Tsujibo *et al.*, 1998).

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In recent years soil inhabitant microorganisms that produce chitinases are considered as potential biocontrol agents against fungi and invertebrate, pests whose physical protective structures are characterized by chitin. Chitinolytic microbes that play important physiological and ecological roles in ecosystems as recyclers of chitin by generating carbon and nitrogen resources may be employed to reduce respective pest based damage of agricultural crops (Kupiec, 1998). Furthermore, chitinolytic enzymes have wide ranging applications such as preparation of pharmaceutically important chitoooligosaccharides, N-acetyl D-glucosamine and single cell protein, isolation of protoplasts from fungi and yeast, control of pathogenic fungi, treatment of chitinous wastes and control of material transmission (Dahiya *et al.*, 2006). Chito-oligomers produced by enzymatic hydrolysis of chitin have been used in agriculture, medical and other industries bringing into use their antibacterial, antifungal, hypocholesterolemic and antihypertensive activities and as food quality enhancer (Bhattacharya *et al.*, 2007).

Biological control of harmful fungi and insects has been dreamt as one of the best solutions representing environmentally friendly ways. There is acute need to design biological control strategies based on local resources so that they may work well in our environments consistently. This study intended isolation of chitinolytic bacteria and their screening on the basis of zone of clearance of chitin degradation. The chitinolytic bacterial diversity has been preserved for further investigation aimed delineating their potential for controlling harmful insects and fungi in environmentally and indoor compatible ways.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from five termites influenced sites. Soil was digged at each location and sampled from depth of 1-3 cm in sterilized bottles, transported to the laboratory and stored at 4°C till further use.

Preparation of colloidal chitin

Colloidal chitin was prepared as described by modifying the method of Souza *et al.* (2009). Five grams of chitin powder from crab shells (Sigma-Aldrich Co USA) were added slowly to 60 ml of concentrated HCl (Merck) and kept at room temperature with mechanical vigorous stirring, for 1 h. The mixture was

then filtered through glass wool and the filtrate was added to a 200 ml of 50% ethanol that was stirred vigorously during the process. The precipitate was transferred to a glass funnel equipped with filtered paper (Whatman No 1) and washed with sterilized distilled water until the colloidal chitin became neutral (pH 7.0). The only modification in the original method made was use of Whatman filter paper No.1 instead of filter paper (80 g/m). The colloidal chitin that was retained on the filter paper was removed with spatula, weighed and stored in the dark at 4°C.

Isolation and Cultivation of chitinolytic bacteria

A select isolation and cultivation medium was prepared according to Souza *et al.* (2009). It contained (g l⁻¹): (NH₄)₂SO₄, 1.0; KH₂PO₄, 0.2; K₂HPO₄, 1.6; MgSO₄ · 7H₂O, 0.2; NaCl, 0.1; FeSO₄ · 7H₂O, 0.01; CaCl₂ · 2H₂O, 0.02 and 1.2% colloidal chitin (w/v). The amount of the colloidal chitin used here represents a modification to the 1.5% employed in the original method. Final pH of the medium was adjusted to 6.0 and sterilized by autoclaving for 15 min at 121°C. One gram of soil was suspended in the select medium and incubated for 5 days at 30°C. The dilutions were prepared by adding 1 ml of the soil inoculated and processed medium culture in 9 ml of distilled water that was subsequently diluted, similarly two times more. Of these dilutions 0.1 ml were spread on the chitin containing select agar medium and the plates were subsequently incubated at 30°C for 96 h. Well separated colonies were streaked on nutrient agar and then again on the selective medium to raise pure cultures. Pure culture of the isolates was preserved on agar slants of the select medium for further use.

Screening and selection of chitinolytic activity

Screening of the bacterial isolates was conducted based on chitinolytic index that was defined as ratio of a clear zone and colony size diameter (ZS/CS). The selective medium was fortified with 0.01% congo red. Growth of the bacterial isolates on this agar medium yielded showed contrasting clear zones around the bacterial colonies.

Morphological and physiochemical characterization of the bacterial isolates

Colonial characteristics like configuration, margins, elevations, consistency, optical density, color and colonial size of the bacterial colonies were recorded, while observing with the help of colony counter. Gram's reaction,

endospore, motility, catalase, oxidase, sulfate, indol, nitrate, citrate, VP, methyl red tests, Starch, Gelatin, Casein hydrolysis as well as various sugars utilization tests of the bacterial isolates were performed as described by Benson (1994) and identified after Holt *et al.* (2000).

RESULTS AND DISCUSSION

A total of five chitin-rich soil samples were collected from different sites in university of the Punjab. A total of 25 chitinolytic bacteria capable of growing fast on the select medium comprising of chitin as sole carbon and nitrogen sources have been isolated from the soil samples processed. Colonies of the bacterial isolates on the nutrient agar medium expressed varied configuration such as round, filamentous, L.form, wrinkled and irregular and spreading. Likewise the isolates' colonies showed variations in their other features while colonies' diameter ranged from 0.63 to 7.42 mm shown in Table I. Out of which three produced vivid zone of clearance of various sizes. The bacterial isolates designated as JF14, JF16 and JF20 yielded zone of clearance to colony size ratios of 2.66, 2.00 and 2.8, respectively. Characterization of extracellular microbial enzymes including chitinases has received increased attention because of their wide range of applications (Bhattacharya *et al.*, 2007). All three strains were Gram positive rods containing endospore. They were positive for catalase, oxidase, nitrate, citrate, VP tests and negative for methyl red and sulfate tests. They were able to hydrolyse casein, starch and gelatin (Table II). All strains were able to utilize different sugars like glucose, xylose, saccharose, sorbitol, mannose, galactose, arabinose, lactose, maltose, fructose, sucrose except mannitol (Table II). Chitin-containing organisms like insects and fungi produce chitinases for their growth and development, whereas bacteria produce chitinases in their saprophytic phase to derive carbon and nitrogen from chitin. An efficient bioconversion of chitin into useful products led to the identification of chitinolytic microorganisms from different habitats including soil. Soil composition and type is the primary determinant for the composition of total and active bacterial communities in soils (Girvan *et al.*, 2003). Poulsen *et al.* (2008) reported that soils amended with chitin, affected the chitinolytic population and also increased chitinase activity in soil.

Since chitinases are able to diffuse through agar, assays to identify chitinolytic bacteria producing chitin-degrading enzymes can be performed by monitoring the degradation of polymeric chitin incorporated into agar medium (Howard *et al.*, 2003). The larger and clear zone of chitin hydrolysis thus can be attributed to the chitinolytic potential of the bacteria. Even though the plate

BACTERIAL CHITINASES

TABLE I.- COLONICAL CHARACTERISTICS OF THE ISOLATED CHITINOLYTIC BACTERIA GROWN ON NUTRIENT AGAR.

Isolate code	Configuration	Margin	Elevation	Consistency	Optical density	Color	Diameter (mm)
JF 1	Filamentous	Wrinkle	flat	Butyrous	opaque	Pale whitish	3.08-5.64
JF 2	Round & filamentous	smooth	flat	Butyrous	opaque	Pale whitish	2.86-7.21
JF 3	L.form	wavy	flat	Butyrous	opaque	Pale whitish	4.78
JF 4	Round	irregular	convex	Butyrous	opaque	Pale whitish	0.63
JF 5	Round	smooth	convex	Butyrous	opaque	Pale whitish	3.14
JF 6	L.form	irregular	flat	Butyrous	opaque	Off white	6.57
JF 7	L.form	irregular	flat	Butyrous	translucent	Pale whitish	4.91
JF 8	L.form	irregular	flat	Butyrous	translucent	Dull white	3.85
JF 9	Wrinkled	smooth	flat	Dry	translucent	Off white	3.36
JF 10	Round	smooth	flat	mucoid	translucent	Off white	2.81
JF 11	Filamentous	branching	convex	Butyrous	opaque	Pale whitish	4.03
JF 12	complex	irregular	umbonate	Butyrous	translucent	Off white	4.98
JF 13	Round with raised margin	smooth	Raised	Butyrous	opaque	Off white	2.22
JF 14	Round	irregular	umbonate	Butyrous	opaque	Pale whitish	7.32
JF 15	Round with raised margin	irregular	Raised	Butyrous	opaque	Off white	2.71
JF 16	Filamentous	irregular	Umbonate	Butyrous	translucent	Off white	3.88
JF 17	Round	smooth	convex	Butyrous	translucent	Off white	2.46-3.97
JF 18	Filamentous	ciliate	flat	Butyrous	translucent	Off white	4.12
JF 19	Round with irregular margin	irregular	Raised	Butyrous	opaque	Light yellow	3.33
JF 20	Round	Smooth	convex	Butyrous	translucent	Off white	1.68
JF 21	L.form	irregular	flat	Butyrous	opaque	Dull white	4.51
JF 22	Irregular & spreading	irregular	flat	Butyrous	opaque	Pale white	7.42
JF 23	Irregular & spreading	lobate	convex	Butyrous	opaque	Pale white	2.36
JF 24	Round	irregular	flat	Butyrous	opaque	Pale white	4.02
JF 25	L. form	Smooth	convex	Butyrous	translucent	Pale white	1.89

TABLE II.- PHYSIOCHEMICAL CHARACTERIZATION OF SELECT CHITINOLYTIC BACTERIAL ISOLATES ^A

Isolates	Gram staining	Endospore staining	Sulfate Test	Growth in anaerobic conditions	Nitrate test	Citrate test	VP test	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Methyl red Test
JF 14	+ve Diplobacilli	+ve Terminal Elliptical	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
JF 16	+ve Streptobacilli	+ve Central Elliptical	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
JF 20	+ve Diplobacilli	+ve Terminal Elliptical	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve

^a. All the three isolates were found positive for glucose, xylose, saccharose, mannose, galactose, arabinose, lactose, maltose, fructose and sucrose utilization tests. While they showed negative results for sorbitol and mannitol utilizations.

method is considered to be a preliminary quantitative test with limited sensitivity, still it represents simple and inexpensive method that helped in separate the chitinolytic bacteria from pool of bacterial diversity. Diversity of the chitinolytic bacteria being reported here is evident from diverse nature of their colony morphologies as well as cell and endospores arrangements. Variety in morphology indicated diversity in bacterial strains.

In conclusion, collection of diverse types of chitinolytic strains may help in the selection of highly efficient chitinolytic microorganisms having varied biotechnological applications. Results of the present study showed that insects inhabiting soil possess variety of chitinolytic bacteria and that following their isolation they might be exploited for different applications varying from biological control of insects, controlled degradation and complete turnover of the chitin substrate.

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TABLE I.- COLONICAL CHARACTERISTICS OF THE ISOLATED CHITINOLYTIC BACTERIA GROWN ON NUTRIENT AGAR.

Isolate code	Configuration	Margin	Elevation	Consistency	Optical density	Color	Diameter (mm)
JF 1	Filamentous	Wrinkle	flat	Butyrous	opaque	Pale whitish	3.08-5.64
JF 2	Round & filamentous	smooth	flat	Butyrous	opaque	Pale whitish	2.86-7.21
JF 3	L.form	wavy	flat	Butyrous	opaque	Pale whitish	4.78
JF 4	Round	irregular	convex	Butyrous	opaque	Pale whitish	0.63
JF 5	Round	smooth	convex	Butyrous	opaque	Pale whitish	3.14
JF 6	L.form	irregular	flat	Butyrous	opaque	Off white	6.57
JF 7	L.form	irregular	flat	Butyrous	translucent	Pale whitish	4.91
JF 8	L.form	irregular	flat	Butyrous	translucent	Dull white	3.85
JF 9	Wrinkled	smooth	flat	Dry	translucent	Off white	3.36
JF 10	Round	smooth	flat	mucoid	translucent	Off white	2.81
JF 11	Filamentous	branching	convex	Butyrous	opaque	Pale whitish	4.03
JF 12	complex	irregular	umbonate	Butyrous	translucent	Off white	4.98
JF 13	Round with raised margin	smooth	Raised	Butyrous	opaque	Off white	2.22
JF 14	Round	irregular	umbonate	Butyrous	opaque	Pale whitish	7.32
JF 15	Round with raised margin	irregular	Raised	Butyrous	opaque	Off white	2.71
JF 16	Filamentous	irregular	Umbobate	Butyrous	translucent	Off white	3.88
JF 17	Round	smooth	convex	Butyrous	translucent	Off white	2.46-3.97
JF 18	Filamentous	ciliate	flat	Butyrous	translucent	Off white	4.12
JF 19	Round with irregular margin	irregular	Raised	Butyrous	opaque	Light yellow	3.33
JF 20	Round	Smooth	convex	Butyrous	translucent	Off white	1.68
JF 21	L.form	irregular	flat	Butyrous	opaque	Dull white	4.51
JF 22	Irregular & spreading	irregular	flat	Butyrous	opaque	Pale white	7.42
JF 23	Irregular & spreading	lobate	convex	Butyrous	opaque	Pale white	2.36
JF 24	Round	irregular	flat	Butyrous	opaque	Pale white	4.02
JF 25	L. form	Smooth	convex	Butyrous	translucent	Pale white	1.89

TABLE II.- PHYSIOCHEMICAL CHARACTERIZATION OF SELECT CHITINOLYTIC BACTERIAL ISOLATES ^A

Isolates	Gram staining	Endospore staining	Sulfate Test	Growth in anaerobic conditions	Nitrate test	Citrate test	VP test	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Methyl red Test
JF 14	+ve Diplobacilli	+ve Terminal Elliptical	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
JF 16	+ve Streptobacilli	+ve Central Elliptical	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
JF 20	+ve Diplobacilli	+ve Terminal Elliptical	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve

^a: All the three isolates were found positive for glucose, xylose, saccharose, mannose, galactose, arabinose, lactose, maltose, fructose and sucrose utilization tests. While they showed negative results for sorbitol and mannitol utilizations.

ANTIMICROBIAL ACTIVITY OF PURE, HEAT EXPOSED AND HERBAL EXTRACTS BLENDED HONEY

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Abstract.- The present study was conducted to investigate antimicrobial nature of a honey sample obtained from Honey Bee Farm of University of the Punjab, Lahore. For this purpose honey samples were taken aseptically directly from the uncapped comb. Microbial inhibitory activity of this sample was tested employing the intact, heat exposed and the honey blended with aqueous extracts of the herbs. Five bacterial species viz *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus coagulans*, *Staphylococcus aureus* and *Escherichia coli* and the three fungal species; *Trichophyton rubrum*, *Trichophyton mentagrophyte*, and *Trichophyton verrucosum* were employed as test microorganisms. Pure (intact) honey sample was effective in controlling all the bacterial and fungal species showing highest growth control for *P. aeruginosa*, with an average growth inhibition zone (GIZ) of 14.66mm. The application yielded a GIZ of 16.50mm for *T. rubrum*. The antimicrobial nature of the honey was found stable at 100°C. The mixture of honey and extracts: *Cinnamomum zeylanicum*, *Curcuma longa*, *Emblia officinalis* and *Acacia concinna* herbs proved to be even more effective than the pure honey. Even lower concentrations of the mixtures proved more effective yielding GIZ upto 30.33mm against the bacteria, and 27.67mm for the fungal species. These results are promising for employing the preparations of honey and herbal extracts for treating skin and wound infections and to use them as natural food preservatives. Further research is required to explore more diverse samples of honey and herbs for their antibacterial potential.

Key words: Antiseptic honey, *P. aeruginosa*, *Bacillus* sp., *S. aureus*, *Trichophyton* sp.

INTRODUCTION

Honey is derived from nectar that is collected from plants and processed by honey bees. Its composition is variable, owing to the differences in plant types, climate, environmental conditions and contribution of beekeepers (Anklam, 1998; Azerredo *et al.*, 2003). However, it has been reported to contain about 200 substances and is considered as an important part of traditional medicines (White, 1979). It had been used for its antibacterial nature since the

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ancient times. It is used for the treatment of some respiratory diseases, skin wounds, burns, asthma, gastrointestinal disorders, skin ulcers, and infected wounds (Russell *et al.*, 1990; Al-Mamary *et al.*, 2002; Orhan *et al.*, 2003; Snow and Manley-Harris., 2004).

Honey has been known to possess antibacterial properties and has an established usage as wound dressing (Molan, 1999; Cooper *et al.*, 2002). Honey has been reported to have an inhibitory effect to around 60 species including aerobes and anaerobes, gram-positive and gram-negative bacteria. An antifungal action has also been observed for some yeast and species of *Aspergillus* and *Penicillium* (Molan, 1992) as well as all the common dermatophytes (Brady *et al.*, 1997). It has been proposed that the healing effect of honey could be due to various physical and chemical properties (Russell *et al.*, 1990; Snow and Manley-Harris, 2004). The inhibitory activities of honey have been attributed to its high osmolality and acidity production of hydrogen peroxide volatiles, organic acid, flavonoid, beeswax, nectar, pollen propolis and sugar composition (Willix *et al.*, 1992; Weston *et al.*, 1999; Shin and Ustunol, 2005). Since the use of antibiotics became wide spread over 50 years ago, microorganisms have developed resistance (Hsueh *et al.*, 2005). Prevalence of antibiotic-resistant microbial species around us has led to attempting re-evaluation of therapeutic use of ancient / traditional remedies.

Besides the honey, herbal medicines have traditionally been the basis of treatment and cure for various diseases. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, and malaria etc. (Dhar *et al.*, 1968; Samy and Ignacimuthu, 1998, 2000). Within the cornucopia of medicinal plants, few possess such a wide spectrum of qualities and medicinal uses as turmeric (root). For countless centuries, many different cultures have used this wonderful, versatile herb *curcuma longa* to treat a myriad of diseases and ailments. The most well known medicinal action of turmeric is its use as a powerful anti-inflammatory, while it also acts as an analgesic, antibacterial, anti-tumor, anti-allergic, anti-oxidant, antiseptic, antispasmodic, astringent, carminative, cholagogue, digestive, diuretic, stimulant, and vulnerary (Frawley *et al.*, 1993).

Embllica officinalis is an antibacterial (Godbole and Pendse, 1960) and anti-inflammatory agent (Asmawi, 1993) and modifies metal induced clastogenic effects (Dhir *et al.*, 1990). *A concinna* pods could be used in treating mycoses (Ahmed *et al.*, 2002), while antimicrobial activity of cinnamon (*Cinnamomum*

zeylanicum), commonly used in the food industry because of its special aroma, has attracted attention of many researchers. It has been demonstrated that *Cinnamomum zeylanicum* oil has an inhibitory effect against meat spoilage organisms (Ouattara *et al.*, 1997). Due to topographical and climatic variations across the country Pakistan is rich in diversity of agricultural cultivation as well as natural flora. Accordingly, many kinds of honeys are available which are natural antibiotic and antioxidant, and remarkable in their composition and antimicrobial nature. The present study was aimed at verifying antimicrobial nature of pure honey obtained from honey bee farm of University of the Punjab, Lahore and its preparations containing extracts of *Cinnamomum zeylanicum*, *Curcuma longa*, *Embllica officinalis*, and *Acacia concinna*. Results of this study will promote application of honey as natural antimicrobial agent. Such efforts are likely to widen the resources for antimicrobial therapies, which at present are acutely required concerning rapid antibiotic resistance development in the microbial pathogens.

MATERIALS AND METHODS

Provision and processing of honey

Honey used in this study was obtained from the Honey Bee Farm of University of the Punjab, Lahore, Pakistan. It was directly taken from an uncapped comb with the help of sterile syringes, transferred to sterilized glass bottles and was then stored in dark place at 23-25°C. For the antimicrobial assays pure (100%) and 15%, 30%, 50% and 75% honey diluted in autoclaved distilled water was applied in these experiments. Honey heated at 100°C for 30 minutes was also employed to determine the effect of temperature on its antimicrobial activity.

Physico-chemical analyses of honey

Non-heated and heated honeys were assessed subjectively for color, granularity, viscosity and aroma. While the honey sample was chemically characterized by performing following tests. Catalase activity was assessed as described by (Benson, 1994). For this purpose few drops of honey were taken on to a clean slide to which 3 drops of hydrogen peroxide were added. Appearance of effervescence indicated catalase activity.

Total carbohydrates were determined by phenol-sulphuric acid method (Dubois *et al.*, 1956) by reacting 2ml of 1000 times diluted honey with standard

amounts of the test reactants under the assay conditions. Dilutions of sucrose were processed like wise and a standard curve was plotted.

Glucose was estimated by O-toluidine method (Hertal *et al.*, 1969). For this purpose 100 μ L of honey diluted in 0.4ml of distilled water was treated with 2 ml of O-Toluidine color reagent. Absorbance was recorded at 625nm. Protein was determined by Biuret method (Gornall *et al.*, 1949) by treating 0.1 ml of honey with 2.00ml of Biuret reagent and reading the absorbance at 540nm. RNA and DNA contents of the honey were estimated after Schneider, (1956) by reacting 2.00 ml of 100 times diluted honey with 2.00 ml of freshly prepared orcinol and diphenylamine reagent, respectively under the given assays' conditions.

Inocula of bacterial species were grown in nutrient broth at 37°C for 24hrs. While the fungi were grown in Sabourand dextrose broth at 37°C for 24 hrs. For each category of the microorganism a loopful of solid growth was inoculated in the 10ml of respective broth. Each microbe was then upscaled with 10% inoculum.

Assessment of antimicrobial activity of honey

Test microorganisms

The bacterial species *Pseudomonas aeruginosa*; *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus coagulans* and *Escherichia coli*, while the dermatophytes; *Trichophyton rubrum*, *Trichophyton mentagrophyte*, and *Trichophyton verrucosum* were employed as test microorganisms in this study. These microbial isolates were obtained from stock culture collection of Environmental Microbiology Lab., University of the Punjab, Lahore. The bacterial and fungal species were revived on nutrient agar and Sabouraud 2% dextrose agar plates, respectively.

Preparation of honey and herbal extracts

Aqueous extracts of four medicinally important plants *i.e.*, *Cinnamomum zeylanicum* (Cinnamon-brak, belonging to Lauraceae family), *Curcuma longa* (Turmeric-roots or tubers), *Emblica officinalis* (Amla) belonging to family Euphorbiaceae and *Acacia concinna* (Sikakai) belonging to family Fabaceae were employed in this study. A given plant material was dried in incubator at 105°C till the consistent weight and ground into fine powder with the help of

grinder. The materials were hermetically sealed in the glass jars until further use.

Forty gms of each plant material was suspended in 400ml of distilled water in a round bottom flask of condenser. The suspension was kept at boiling and then filtered through Watmann filter paper No. 1. The filtrate was transferred to an open vessel and allowed to evaporate till dryness. Weight of the dried extracts were determined and then stored in air tightened bottles. The dried extracts were then used to represent their 1 through 5% mixtures with honey. The preparations were processed for determination of their antimicrobial effects.

Discs of 10mm diameter prepared from Whatmann filter paper No. 1. were used for loading a given application. After sterilizing the discs by autoclaving, they were kept in sterile Petri-plates. Then 10 μ l of honey, its given dilution or herbal preparation was dispensed on labeled discs with the help of a micropipette. The loaded discs were left for drying under sterile condition. Then 100 μ l cultures of bacterial and fungal microbes were spread on nutrient and Sabouraud agar plates, respectively with sterile cotton swabs. The discs were then placed on the swabbed plates at equal distance and gently pressed. Six discs were placed on each plate and incubated at 37°C for 24 hrs.

After the incubation, the growth inhibition zones were measured with a metric ruler to the nearest fraction of a millimeter. Diameters of the zones were then recorded for further calculations. Results were expressed as mean \pm S.E.M. ANOVA tests were performed for determining significance between differences of respective experiments.

RESULTS AND DISCUSSION

Intact and the heated honeys appeared different from each other. Untreated honey has granular, less viscous and was of light amber color with distinct aroma. While the heat exposed honey attained a viscous and darker look with strong aroma and lost the granular appearance. Chemical analysis showed that the honey contained 2591.10 μ g/ml, 194 μ g/ml and 13.27 μ g/ml of total carbohydrates, glucose and protein contents, respectively. The sample was found to contain 0.994 μ g/ml, 1084.531 μ g/ml of RNA and DNA, respectively.

Application of intact (pure honey) yielded a growth inhibition zone (GIZ) of 14.66 \pm 0.10mm diameter for *P. aeruginosa*, *B. cereus* appeared least susceptible to the pure honey with average GIZ of 11.28 \pm 0.14mm (Table I).

Heated honey yielded comparable and variable results for the different bacterial species (Table I). The growth inhibiting effect of heated honey reduced and increased for the *P. aeruginosa* and *B. cereus*, respectively. Regarding antifungal activity of the pure honey, *T. rubrum* was strongly inhibited with GIZ of 16.50 ± 0.28 mm. Whereas *T. mentagrophyte* appeared least inhibited with GIZ of 14.16 ± 0.44 mm (Table II). In case of fungal test organisms it was found that growth inhibiting effects of the heat exposed honey reduced for all the species as compared to the effects of pure honey (Table II).

TABLE I.- ANTIBACTERIAL EFFECTS OF INTACT (A) AND HEATED (B) HONEY AGAINST THE BACTERIAL SPECIES.

Test Bacterium	(A)	(B)
<i>Pseudomonas aeruginosa</i>	$14.66^a \pm 0.10$	$12.83^a \pm 0.356$
<i>Bacillus cereus</i>	$11.28^b \pm 0.148$	$13.33^b \pm 0.607$
<i>Bacillus coagulans</i>	$11.50^b \pm 0.325$	$12.28^{ab} \pm 0.607$
<i>Staphylococcus aureus</i>	$12.41^b \pm 0.300$	$12.28^{ab} \pm 0.148$
<i>Escherichia coli</i>	$12.65^b \pm 0.351$	$12.16^a \pm 0.641$

Values represent diameters of Growth Inhibition Zones (GIZ) in mm and are means \pm S.E.M. of triplicates. Values not sharing same alphabets are significantly different from each other with in a column. Single factor analysis of variance.

TABLE II.- ANTIFUNGAL ACTIVITIES OF PURE (A) AND HEATED (B) HONEY AGAINST THE FUNGAL SPECIES.

Test Fungus	(B)	(A)
<i>Trichophyton rubrum</i>	$16.50^a \pm 0.289$	$13.96^a \pm 0.0260$
<i>Trichophyton mentagrophyte</i>	$14.16^b \pm 0.441$	$12.00^b \pm 0.289$
<i>Trichophyton verrucosum</i>	$14.5^b \pm 0.289$	$12.83^b \pm 0.441$

Values are represent GIZ and mean \pm S.E.M of triplicates. Values not sharing same alphabets are significantly different from each other with in a column.

All the aqueous dilutions of pure as well as heat exposed honey failed to show antimicrobial activity against any bacterial and fungal test organism reported in this study. Pure honey mixed with the herbal extracts was also applied against the test microorganisms. Honey mixed with *E. officinalis* proved very effective in inhibiting *B. cereus*, as its 5% extract's inclusion yielded a GIZ of 18.00 ± 0.57 mm, as compared to a value of 11.28 in case of pure honey (Tables I, III). The test organisms *P. aeruginosa* and *B. coagulans* did not show influence of the herbal application. While *S. aureus* and *E. coli* species, which showed

growth inhibition to the pure honey expressed complete resistance to the blending of honey and *E. officinalis* extract (Table III).

TABLE III.- ANTIBACTERIAL EFFECTS OF PURE HONEY CONTAINING DIFFERENT AMOUNTS (%) OF AQUEOUS EXTRACTS OF *E. OFFICINALIS* (A). *C. ZEYLANICUM* (B) AND *C. LONGA* (C).

Test Bacterium	Extract %	A	B	C
<i>Pseudomonas aeruginosa</i>	1	R	14.67±0.33	20.33 ^a ±0.333
	2	R	R	22.33 ^b ±0.333
	3	7.5 ^a ± 0.333	13.33± 0.333	24.67 ^c ±0.333
	4	10.67 ^b ±0.337	R	27.33 ^d ±0.667
	5	12 ^c ± 0.00	12±0.578	30.33 ^e ±0.882
<i>Bacillus cereus</i>	1	R	12.33±0.667	R
	2	R	R	R
	3	R	13.67±0.337	R
	4	16.00 ^a ±0.578	R	R
	5	18.00 ^b ±0.578	15.33±0.37	R
<i>Bacillus coagulans</i>	1	R	R	R
	2	R	R	R
	3	R	R	R
	4	R	R	R
	5	12.00±0.578	R	R
<i>Staphylococcus aureus</i>	1	R	R	R
	2	R	R	R
	3	R	R	R
	4	R	R	R
	5	R	R	R
<i>Escherichia coli</i>	1	R	12.33±0.5578	R
	2	R	R	R
	3	R	13.67±0.333	R
	4	R	R	R
	5	R	15.33±0.333	R

R= the microbe was found resistant to the respective applications and all the bacterial species were resistant to the preparation containing extract of *A. concinna*.

Values are represent GIZ and mean ± S.E.M of triplicates. Values not sharing same alphabets are significantly different from each other with in a column.

Application of 5% *C. zeylanicum* extract in honey appeared effective for *B. cereus* and *E. coli* yielding GIZ upto 15.3mm. The application of honey mixed with *C. longa* extract strongly inhibited but only the bacterium *P. aeruginosa* in a dose dependant manner, yielding GIZ upto 30.33mm for 5% extract. This extract application caused 107% increase in the growth inhibition effect of the honey (Tables I, III).

In case of *A. concinna* all the test bacterial species appeared resistant to all the %ages of the extract applications. Blending of the herbal extracts, in general, showed better growth effects for the fungal test organisms. In case of the herb *E. officinalis*, inclusion of 5% aqueous extract in the honey yielded GIZ of 26.67, 20.74 and 23.33mm against *T. rubrum*, *T. mentagrophyte* and *T. verrucosum*, respectively. Likewise the extract of *C. zylanicum* proved to be effective for all the fungi yielding GIZ of 27.00mm against *T. verrucosum*. For *T. rubrum* 1% concentration was found more effective than 5% yielding GIZ of 26.78 and 23.22mm, respectively (Table. 4). Application of *C. longa* proved effective for the fungal species in every concentration. However, it gave GIZ of 27.67mm for *T. verrucosum*. Honey mixed with *A. concinna* also found to be effective against all the fungi but it inhibited *T. rubrum* more with GIZ of 25.00 mm diameter (Table IV). Overall these preparations were found more effective against the fungal species than the bacteria.

TABLE IV.- ANTIFUNGAL ACTIVITIES DIFFERENT AMOUNTS (%) OF HERBAL EXTRACT MIXED WITH HONEY AGAINST THE FUNGAL SPECIES.

Test fungus	Extract %	<i>E. officinalis</i>	<i>C. zylanicum</i>	<i>C. longa</i>	<i>A. concinna</i>
Trichophyton rubrum	1	R	26.78±0.400	14.67 ^a ±0.333	13.67 ^a ±0.887
	2	R	R	16.67 ^{ab} ±0.667	15.67 ^b ±0.882
	3	19.11 ^a ±0.110	19.03±0.607	19 ^b ±0.88	18.33 ^b ±0.882
	4	21.87 ^b ±0.294	R	21b ^c ±0.667	20 ^c ±0.578
	5	26.67 ^c ±0.193	23.22±0.910	23 ^c ±0.88	25 ^d ±0.578
Trichophyton mentagrophyte	1	R	23.22±0.233	13.33 ^a ±0.333	R
	2	R	R	19.67 ^b ±0.337	R
	3	14.33 ^a ±0.333	21±0.578	21.44 ^c ±0.728	12.33 ^a ±0.333
	4	17.67 ^b ±0.333	R	24.67 ^{cd} ±0.337	21.33 ^b ±0.333
	5	20.74 ^c ±0.375	22.33±0.667	26.67 ^d ±0.333	24 ^c ±0.578
Trichophyton verrucosum	1	R	24.33±0.667	12 ^a ±0.578	11.83 ^a ±0.929
	2	R	R	16.33 ^b ±0.337	15 ^b ±0.578
	3	16.33 ^a ±0.193	17±0.578	18.67 ^b ±0.337	16.67 ^{bc} ±0.337
	4	19.89 ^b ±0.110	R	21.00 ^c ±0.578	18.33 ^c ±0.337
	5	23.33 ^c ±0.889	27±0.578	27.67 ^d ±0.882	23.33 ^d ±0.337

R=the microbe was found resistant to the respective applications.

Values are represent GIZ and mean±S.E.M of triplicates. Values not sharing same alphabets are significantly different from each other with in a column.

Honeys have long been recognized for their antimicrobial activity against bacteria, moulds and yeasts. High osmotic pressure, low water activity, low pH, low redox potential of honey, hydrogen peroxide and other phytochemical factors might contribute to the honey antimicrobial nature. Their relative importance depends on the sensitivity of the species and the level of additional factors in an

honey preparation (Molan, 1992a,b). In the present study, failure of antimicrobial activities of diluted honey is attributable to effective osmolarity of pure honey. *P. aeruginosa* was found to be the most susceptible bacterium inhibited by pure honey. *P. aeruginosa* is recoverable from skin wounds, particularly those related to burns. It causes a variety of systemic infection, particularly in victims of severe burns (Yau *et al.*, 2001).

Antibacterial activity of the honey sample against *P. aeruginosa* may prove of importance for the development of ointment for the treatment of skin wounds. Cooper *et al.* (2002) tested the sensitivity of 171 strains of *P. aeruginosa* isolated from burn and found similar sensitivity of honey for all strains with MIC below 10% (v/v). While in this study diluted honey failed to show any inhibition against the bacterial as well as fungal species. Nzeako and Hamdi (2000) also reported that inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* did not occur with diluted honey. Honey's hygroscopic nature is associated with its antimicrobial effects. Further, dilution(s) of antimicrobial chemical ingredients' of a honey sample below their MIC, might have no effect against growth of microbes.

Potent growth inhibiting effects of honey against the dermatophytes *Trichophyton rubrum*, and *I. mentagrophyte*, is of importance. The present results showed that the antimicrobial substances in honey can withstand temperatures and are heat stable at 100°C. Honey heated at 100°C was found to be inhibitory for all bacterial and fungal species tested. The antimicrobial activities of the herbal extract-honey mixtures appeared highly significant as compared to the effects of pure honey. Conclusively, locally produced honey in general and its herbal extract preparations in particular had promising antimicrobial activities against a range of microbes. Further research is required to determine that to what extent such preparations are effective in controlling concerned microbial pathogens.

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Some Abstracts

SECTION - I**CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS,
PHYSIOLOGY, TOXICOLOGY****ANALYSIS OF TP53 “HOT SPOT” MUTATIONS IN PAKISTANI
COLORECTAL CANCER PATIENTS BY RESTRICTION FRAGMENT
LENGTH POLYMORPHISM (RFLP) ANALYSIS**

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TP53 is the most commonly mutated gene in human cancers. More than 50% of human cancers show mutation in this gene. Some of the codons of this gene in the DNA binding domain frequently show mutations in almost all type of cancers including colorectal cancer, so these codons are called hot spots. It is generally believed that more than 30% mutations of this gene occur in these hot spots in various human cancers. In order to find mutations in these hot spots among Pakistani colorectal cancer patients, tissue and blood samples were collected. DNA was isolated and the regions containing the hot spots were amplified using naturally occurring and artificially created restriction sites. These amplified products were then restricted with appropriate enzymes. In case of all the hot spots studied, mutant allele was represented by uncut band, while the normal was always represented by two restricted fragments. Samples showing mutations were confirmed by sequencing.

**STATUS OF *KIRSTEN RAS VIRAL ONCOGENE HOMOLOG (K ras)* CODON 13
MUTATIONS IN PAKISTANI CRC PATIENTS**

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Colon cancer is the third most frequently detected cancer worldwide, accounting for close to 1 million new patients every year. Colon cancer has the third highest mortality rate, following lung and breast cancer. Oncogenic mutations in *Kirsten ras viral oncogene homolog (K ras)* is one of the most common genetic alteration in CRC. *K ras* is involved in G protein-mediated signal transduction. It has constitutive GTPase activity, which is lost when the gene is mutated most commonly at codons 12, 13 and 61 (contributing 75%, 9% and 5% of the total *K ras* mutations, respectively). Therapies that target the Ras proteins signaling pathways are therefore very valuable in treating tumors containing Ras mutations. Different geographical conditions and dietary carcinogens have been shown to induce characteristic *K ras* mutations. Present study

aims to analyze the status of *K ras* codon 13 mutations in Pakistani CRC patients. It will be relevant for both the molecular diagnosis of cancer and for the patient prognosis.

ANEMIA IN PREGNANT WOMEN OF PESHAWAR NWFP

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Maternal mortality continues to be a major health problem in the developing world. Estimates of maternal mortality from anemia range from more than 194 per 100,000 in Pakistan. To analyze anemia in pregnant women, blood was taken, randomly from the 200 pregnant women attending the antenatal clinic of the Khyber Teaching Hospital and Government Hashtnagri Hospital, Peshawar Pakistan. A questionnaire was filled by direct interview from all the women included in this study. Out of 200 women 39% women were found to be anemic. Among them, 11% had Hb 7-10 g/dl while 28% had Hb 10-11 g/dl. None of the women were found to be severely anemic. The mean Hb of anemic women was 10.52 g/dl and that of non -anemic women was 11.64 g/dl, showing that the situation was not alarming. Distribution pattern of Hb values among the women suggesting that majority of non-anemic women had Hb value in the range of 11.0-11.9 g/dl while majority of the anemic women had Hb value in the range of 10-10.9 g/dl which is considered as mild anemia. The effect of different socio-economic status on the prevalence of anemia ($\chi^2 = 7.053$) showed that a significant association between prevalence of anemia and socio-economic status exist. The mean Hb in lower class was 10.87 g/dl, lower middle class was 11.20 g/dl and middle class was 11.96 g/dl. The results suggest that teenage pregnancy, 3rd stage of pregnancy, grand multiparity, low socio-economic status and illiteracy are milestones in relation to anemia in pregnant women.

EPIDEMIOLOGICAL STUDIES AND MOLECULAR DIAGNOSIS OF GIARDIASIS IN BOVINE

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Epidemiological studies were undertaken at Military dairy farm, Government dairy Farm, Gawala dairy Colonies and House hold dairy in Lahore under different managemental and climatic conditions of four different areas, s at Lahore. Infection rate amongst the Calves, Cattle and Buffaloes were (50.27%), (28.05 %), and (26.11 %) respectively. In Calves, highest prevalence was recorded in Government. Dairy farm

(68.33%), and the lowest (34.44%) were recorded in Household dairy. Overall highest (61.6%) season wise prevalence was recorded during autumn and the lowest (34.1%) was recorded during winter. ($P < 0.05$) was noted. In Cattle, the highest (41.67%) prevalence was recorded at Government Govt. dairy farm and the lowest (15%) was recorded in Household dairies. Similarly in buffaloes, the highest (40.55%) prevalence was recorded at Government Dairy Farm and the lowest prevalence (12.77%) was reported in Household Dairies. The insignificant difference was recorded ($P > 0.05$). It was observed that the higher infection rate was recorded in younger than older animals. Female were more commonly affected than male.

CLONING OF DIFFERENT LENGTHS OF TOXIC DOMAINS OF Cry1C GENE FROM HD137 STRAIN OF *BACILLUS THURINGIENSIS*

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Gram-positive, endospore forming, soil born bacterium *Bacillus thuringiensis*, produces a parasporal protein toxin called as insecticidal crystal protein (ICP) are selective biodegradable insecticides has alternatives to synthetic chemical insecticides. In the genes coded insecticidal proteins are located on plasmids or chromosomes. *CryI* genes responsible for lepidopteron larval toxicity. Among them *cry1C* is the most toxic crystal protein against mosquitoes and Armyworms has been reported so far. The cytolytic- δ endotoxin gene *cry1C* 3.6 Kb was amplified from genomic DNA of HD137 by PCR using primers were designed from the sequence of *cry1C* gene accession number X07518. The PCR amplified 3.6 kb product from strain was further cloned in pTZ57 cloning vector. The cloning of 3.6Kb genes was confirmed by restriction analysis by Restriction endonucleases. Then different lengths of *Cry1C* gene were cloned using the full length gene as template.

EFFECT OF EGG ADAPTED GAMETOCYTIC VACCINE (LOCAL ISOLATES) ON LESION SCORE IN COCCIDIOSIS IN POULTRY

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An egg adapted gametocytes (Local isolates) *E. tenella*, vaccine(s) were used against coccidiosis in chickens. A total of two hundred chicks were utilized in this study.

On day fifth of chicks they were divided into four groups, fifty chicks in each group and their distribution was as; Group-I, Group-II, Group-III and Group-IV and were administered vaccine(s) for immunization orally viz; Vaccine I (gametocytes), to Group-I of chicks, Vaccine II (gametocytes inactivated) to Group-II, Vaccine III (gametocytes sonicated inactivated) to Group-III and Group-IV was served as control given normal saline. On day 15th post-immunization chicks were challenged with 60,000–70,000 sporulated oocysts of mixed species of *Eimeria*. On day 21st post vaccination birds were subjected to postmortem and their lesions score were recorded. A maximum of 46 (92%) birds having lesions in intestine and caeca of Group-IV were observed while a minimum of 17 (34%) birds having slight lesions in intestine and caeca were observed in Group-III. There was non-significant difference ($P>0.05$) in lesions score of Group-I, II and IV. Lesions scores in Group-III were significantly different ($P>0.05$) from Group-I, II and IV. It is concluded that on the basis of lesions score the egg-adapted vaccine saved the chicks against coccidiosis in challenge.

GR24 IMPACT ON GENE EXPRESSION OF SOLOPATHOGENIC STRAIN OF *SPORISORIUM REILIANUM*

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Sporisorium reilianum f.sp. *zeae*, a basidiomycetous fungus belonging to Ustilaginaceae, is the causal agent of the maize head smut disease. Its pathogenicity is initiated by fusion of two compatible yeasts which give rise to the formation of dikaryotic pathogenic hyphae. In addition, pathogenic dimorphic diploid strains (Solopathogen) can be formed without mating. Strigolactones are trace molecules in plant root exudates that are perceived by some fungi at subnanomolar concentrations. In order to investigate the mechanism of infection, we analysed the transcriptome of some biotrophic related genes of the fungus in response to an analogue of Strigolactones, GR24 during the previous phase of infection. A burst of cell respiration was observed at 1h post addition of GR24 (+10 %). This induction decreased at 5 and 8 hours to (-6%) and (-2%) of induction respectively. In this study, the expression level of biotrophic genes was analyzed with some candidate genes related. QRT-PCR data show that treatment of solopathogenic strain of *S.reilianum* with GR24 causes a rapid increase (1h post addition) in gene expression of the fungus involved in cell respiration and cell wall development as Cytochrome-c oxidase activity, succinate dehydrogenase, polygalacturonase activity, actin and myosin during the previous step of infection. This result points out that Strigolactones could have a large incidence in the formation of the rhizosphere and could play an important role in fungi growth promotion and plant pathogenesis.

SEROPREVALANCE AND RISK FACTORS OF TOXOPLASMOSIS AMONG PREGNANT WOMEN IN DISTRICT KOHAT

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180 blood samples were collected randomly to determine the seroprevalance, risk factors and possible contaminations routs in pregnant women of District Kohat. IgM was detected by ELISA for seroprevalance and clinical symptoms associated with investigations of seropositivity of *Toxoplasma gondii*. Total 14.4% (26/180) was confirmed IgM positive in the current study. There was significant difference of seropositivity between inhabitants of rural and urban areas 21.27% and 6.97% respectively. An increased consumption of undercooked meat 21.53% and unwashed vegetables or fruit 25% were observed in serologically positive women. A significant difference between women consuming unpasteurized milk was found $p=0.009$. Increased seropositivity was also found in pregnant women with consumption of municipal and uncontrolled water (spring water) supply. Clinical symptoms like anaemia, fatigue, fever, muscle weakness and headache were most common among the seropositive patients. Community especially pregnant women are at great risk to toxoplasma infection in the study area due to unhygienic conditions.

CRYOPRESERVATION OF BUFFALO (*BUBALUS BUBALIS*) SEMEN IN BIOXCELL® EXTENDER

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This experiment was designed to compare commercially available extender Bioxcell® with *tris*-citric-egg yolk extender for post thaw quality of buffalo semen. Semen was collected from five adult Nili-Ravi buffalo (*Bubalus bubalis*) bulls of similar age group with artificial vagina (at 42°C) for three weeks (replicates). Qualifying ejaculates from each buffalo bull were divided into two aliquots and diluted (at 37°C having 50×10^6 spermatozoa/ml) in *tris*-citric-egg yolk or Bioxcell® extender. Diluted semen was cooled to 4°C in 2 hours, equilibrated for 4 hours and filled in 0.5 ml straws. Semen straws were kept over liquid nitrogen vapours (5cm) for 10 minutes. Straws were then plunged and stored into liquid nitrogen (-196°C). After 24 hours of storage, semen straws were thawed at 37°C for 30 seconds to assess sperm quality viz; sperm motility, viability, plasma membrane integrity, normal apical ridge and abnormalities (head, mid

piece and tail). Post-thaw percentage of sperm motility (45.3 ± 1.1 , 45.0 ± 1.4), viability (66.2 ± 1.1 , 64.4 ± 1.3) plasma membrane integrity (60.4 ± 1.2 , 59.2 ± 1.4) and normal apical ridge (82.9 ± 0.5 , 80.7 ± 0.5) did not differ ($P > 0.05$) in *tris*-citric egg yolk and Bioxcell® extender, respectively. Similarly, sperm abnormalities of head (1.20 ± 0.1 , 1.20 ± 0.1), mid piece (0.67 ± 0.1 , 0.87 ± 0.1) and tail (11.7 ± 0.2 , 11.6 ± 0.3) remained similar ($P > 0.05$) in *tris*-citric egg yolk and Bioxcell® extender, respectively. It is suggested that commercially available Bioxcell® may be used for the cryopreservation of buffalo semen with an equal efficiency to *tris*-citric egg yolk extender.

DNA-BASED CHARACTERIZATION OF PHEASANTS OF NORTHERN PAKISTAN

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Pheasants are group of large birds in the order Galliformes, family Phasianidae. Galliformes is a large and diverse order comprising approximately 70 genera and 250 species. Taxonomists have always considered the pheasants to be more closely related to the Old World partridge, quail, and francolin species than to any other Galliformes group. Pheasants are economically important as game birds and sport hunting. During the present study, genetic diversity of six male and female species *i.e.*, Koklass, Monal, Western Tragopan, Kalij and the Cheer pheasant) were studied using ten pheasants specific simple sequence repeat primers (SSR). The results revealed low-medium amount of genetic variability (GD ranging from 4 – 57%) among the Pheasants. Maximum genetic diversity (GD= 57%) was estimated among one comparison (Himalayan monal female -- Blue peacock male), which was closely followed by genetic distance of 54% among one comparison (white crested kalij male – western Tragopan female). Two comparisons (Himalayan monal male – cheer pheasant female and Western Tragopan male -- Cheer pheasant female) showed minimum genetic diversity (GD = 4%). Genetic distances between the pheasant's genotypes supported their ancestral development. Preliminary phylogenetic studies, using DNA based markers (SSR), revealed that three species viz, Koklas, Himalayan monal and Blue peacock belong to same statistical clusters (clusters A, C and D respectively). Present study yields the first documented report of the genetic characterization of the commonly found pheasant species in Pakistan, using DNA based markers.

UV-INDUCED MUTATIONS IN *SERRATIA MARCESCENS* FOR ENHANCED PRODUCTION OF PRODIGIOSIN

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The strain of *Serratia marcescens* BDCS-N-S1, a wild-type prodigiosin-producer, was subjected to physical mutation involving treatment by UV-irradiation. The UV lamp of 254 nm intensity was used for irradiating bacterial cells in a dilution of 10^{-6} cfu ml⁻¹ for 30 to 120 sec. Diluted cells were exposed to UV-irradiation from a distance of 30 cm. The red-pigmented colonies, appearing on peptone glycerol agar, confirmed the presence of prodigiosin (anti-fungal antibiotic) in wild-type as well as in survivor colonies. A considerable number of survivors with enhanced prodigiosin production were retained which showed higher OD values (>2.4 at 535 nm; >2.35 at 470 nm) than their parents (2.12 at 535 nm; 2.02 at 470 nm) both in the acidic and basic forms. Prodigiosin was well-expressed in all the mutants after an incubation period of 72 hour and significant variations were observed among UV-irradiated survivors and their parent. The optimized conditions resulted in four hyper-producing mutants (UVM¹, UVM⁴, UVM⁷ and UVM¹⁰) with many-fold increase in prodigiosin expression. These promising mutants could be used as bio-control agent against fungal diseases of rice.

OSTEOBLASTOGENIC EFFECT OF A LOCAL HERB, *CISSUS QUADRANGULARIS*

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Osteoblastogenesis is a multi-step process that commences with the proliferation of mesenchymal stem cells (MSCs) and their subsequent differentiation into osteoblasts. This process is a requisite for curing numerous bone diseases and conditions. Currently, various synthetic agents are being used to treat these ailment but with numerous side effects. Ayurvedil literature and various scientific studies have described the use of herb *Cissus quadrangularis* and its extracts in treatment of osteoporosis and fractures of bones. The aim of this study is to determine the effect of extract of this herb on the differentiation of MSCs into osteoblasts. Mesenchymal stem cells will be isolated from rat bone marrow. The ethanol extract of herb *Cissus quadrangularis* will be prepared. Cytotoxic effect of various concentration of this extract on MSCs will be determined. MSCs will be then induced to differentiate into osteoblasts using herbal extract or

dexamethasone (positive control). Osteoblastogenesis will be assessed by quantification of expression of certain osteoblast related genes and by alkaline phosphatase. assay. Control and experimental results will be compared statistically, to reveal the effect of *Cissus quadrangularis* on osteoblastogenesis.

**ASSOCIATION OF ELECTROPHORETICALLY RESOLVED LOW
MOLECULAR WEIGHT PROTEIN FRACTIONS WITH SENILE DEMENTIA
IN ELDERLY PEOPLE**

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The study was designed to investigate protein fractions and their relationship with dementia in elderly people. Serum blood samples of volunteers with more than 65 years of age were collected from psychiatric clinic in Lahore. A Mini Mental State Examination (MMSE) was conducted to screen the senile dementia. Later on, these subjects were categorized on the basis of MMSE score into four groups *i.e.* normal, mild, moderate and severe dementia subjects. Severe dementia subjects were also receiving multivitamins and protein supplements since one month. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-P AGE) was employed for studying protein profile of control and demented groups. Gels were photographed and their images were stored by Gene Genius Bio-imaging Gel Documentation System, which provided the data of molecular weights and percent raw volumes for each of the fractions. The data was analyzed statistically using student t-test in finding the enhancement or reduction and appearance or disappearance of particular protein fractions for comparisons among the demented and the control subjects. Eight protein fractions ranging 66-14kDa were detected in all of the study groups. Significant reductions were observed in 66,54,20 and 14kDa protein fractions in mild, moderate and severe dementia subjects when compared with control group. No significant alterations in 40, 33 and 25kDa protein fractions were observed in mild dementia subjects, however, the fractions declined significantly in moderate and severe dementia subjects as compared to control group. Fraction of 23kDa showed significant elevations in mild and severe dementia subjects with no variations in moderate dementia group as compared to control group. There was a progressive decline in most of the serum proteins with advancing stage of senile dementia but in severe dementia group, protein profile was closer to control group. It might be due to the supplemented diet provided to severe dementia people, indicating that well balanced diet if provided for longer time periods might be helpful either in treating dementia or preventing its manifestation before its onset.

INHIBITION OF CSC SPECIFIC miRNA RESULTS IN DIFFERENTIATION OF CSC INTO CARDIOMYOCYTES

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Several studies suggest that miRNA have important roles in the development of the heart and cardiac function. Cardiomyocytes can be derived from hESC and human cardiomyocyte progenitor cells by treatment with activin A (Acv), bone morphogenic protein (BMP) or transforming growth factor, beta receptor III (TGF- β III). We hypothesized that inhibition of specific miRNA in mouse cardiac stem cells (CSC) and CSC with GAT A-4 (CSCG) could induce differentiation in cardiomyocytes by activation these target genes. We studied 569 unique miRNAs probes in mouse heart cells (MHtC), CSC and CSCG and identified high expression miR 762, 21 and 31 in CSC and CSCG compared to MHtC. CSC and CSCG were cultured in matrigel and anti-mRNAs were transfected with oligofectamine reagent twice (3 and 15 days) after plating cells. We inhibited miR-762; 762+21; 762+31 and 762+21+31, which present as target genes activin A, BMP and TGF- β receptors. The target genes as TGF β III, activin A (receptors type-IE, II-like-I, 2a) BMP (receptors type 2), as well as Smad-4 and Dicer were analyzed by RT-PCR, 30 days after the last transfection, also cardiac sarcomeric proteins (*ex* and p-MHC, MLC-2a, MLC-2v and cTNI). Beating cells were recorded on Confocal microscopy. As a positive control the cells were treated with activin A and BMP-4 cytokines and also compared to MHtC expression. The anti-sense was tested 1, 5, 10, 50 and 100nM. After 72hs of the treatment the target genes were analyzed by RT-PCR. In CSC and CSCG the TGF β III, activin AR, BMPR and Smad4 expression increased up 50nM and beginning inhibition with 100nM concentration. The maximum miRNA expression inhibition was observed 6hs after the treatment, and 24hs the miR expression was similar of the control cells, however the target genes expression were maintain increased. As expected cytokines treatment increased the target genes and signaling pathway increasing Smad-4. In CSC the miR-762, 762+21 and 762+21+31 anti-sense increased the target genes TGF β III, AcvR (AcvR 2a and like-I), but not BMPR. Furthermore, increase the transcription factor Smad-4 and MLC-2a. a-MHC expression increased with 762+21 anti-sense association. In CSCG the 762+31 and 762+21+31 inhibition induced the genes, including TGF β III, AcvR (2a and BI), but not BMPR. Also, increase Smad4 and MLC-2a and a-MHC (with 762+31 association). The results show that CSC and CSCG differentiate in cardiomyocytes by activation of TGF β III and AcvR signaling pathway, but no BMPR. Also CSC differentiation was most activated by inhibition miR-762+21, while in CSCG by inhibition miR-762+31.

QUANTITATIVE EXPRESSION ANALYSIS OF CUS OPERON IN *KLEBSIELLA PNEUMONIAE* AT TRANSCRIPTIONAL LEVEL THROUGH REAL TIME PCR

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Klebsiella pneumoniae has some genetic elements resembling, on homology basis, cus operon present in *Escherichia coli* genome and has been reported as taking some part in copper resistance mechanism. This operon consists of two divergently transcribed parts, regulated by a same bidirectional promoter. One part, comprising of two genes namely cus S (copper sensor) and cus R (regulator), plays regulatory role. While the other transcriptone encodes four structural proteins and is comprised of *cus C*, *cus F*, *cus B* and *cus A* genes. The expression of the both parts of the operon is measured at transcriptional level in *Klebsiella pneumoniae* through quantitative real time PCR. This study has been conducted in absence and presence of copper in both aerobic and anaerobic conditions. It has been found that this operon plays a significant role for copper resistance, under anaerobic conditions.

INDUCTION OF PLURIPOTENT STEM CELLS FROM HUMAN FIBROBLASTS AND HEPATOCYTES BY ECTOPIC EXPRESSION OF DEFINED TRANSCRIPTION FACTORS USING ADENO ASSOCIATED VIRUS (AAV)

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Production of induced pluripotent stem cells (iPSC) is a breakthrough in biological sciences. iPSC generation has great potential for basic and clinical sciences. There is a possibility of nuclear reprogramming using somatic cell nuclear transfer technology and fusion of embryonic stem (ES) cells with mature cells but potential of these approaches was hindered by technical complications and ethical concerns. Main objective of iPSC production is the derivation of "customized" embryonic stem cells for patient specific cell treatment. An attempt has been made to induce *in vitro* reprogramming of human fibroblasts and hepatocytes by ectopic expression of four transcription factors, Oct4, Sox2, Klf4 and c-Myc by Adeno Associated Virus (AAV). Exogenous expression of these genes was studied by PCR. iPSC production was also analyzed with or without expression of c-Myc (a transcription factor). ES cells like colonies were observed in both fibroblasts and hepatocytes. iPSC colonies were confirmed by immunostaining with ES cell markers (Oct-4, SSEA-4, TRA-1-60 and

TRA-1-81). iPSC colonies were also generated using Oct-4 promoter and Geneticin (G480) as selectable marker. Our results indicate that iPSC can be generated by ectopic expression of three TF in mature human cells. There is further need of *in vivo* study to test these iPSC for germ line transmission and teratoma formation and *in vitro* differentiation into all the three germ layers.

EFFECT OF ENDOSULFAN AN ORGANACHLORINE INSECTICIDE ON THYROID GLAND OF RABBIT

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These studies were carried out to identify the level the loss carried by the application of endosulfan as an endocrine disruptor on the thyroid gland of the rabbits living in the agricultural lands and face continuous spray of the insecticides want of good crop. About 50 animals were used in this study with the mean body weight 1400 ± 75.5 grams. The animals were initially kept in animal house measuring 10x4 sq.ft. for acclimatization in the captive environment. All the animals were divided into four groups of 12 animals each. One group was used as control remaining three groups were kept in three different cages measuring 3 x 3 x 1.5. doses of the insecticides were prepared as to the group for a period of 15 days. During the experimental period the animals exposed to higher dosage showed the clinical signs of intoxication in both males and females throughout the 15 day experiment, these signs included: 'tremor', generalized convulsion, salivation. Increased respiratory rate, hyperactivity and reddish nasal discharge in number of animals. Swelling of hind limbs were also observed and these signs were persisted in number of cases throughout the experiment. Histological examination of thyroid gland reveal that the damage caused in the group exposed to lowest dose was less as compared to the higher dose. Hence it is concluded that the effect of the insecticide is dose dependent.

MOLECULAR CHARACTERIZATION OF *cry10* CRYSTAL PROTEIN GENE FROM LOCAL ISOLATES OF *BACILLUS THURINGIENSIS*

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Every year 2,400 million about 40% of the world's population are estimated to be affected by malaria and about 20 million people are infected every year by dengue virus transmitted by *Aedes* mosquitoes with about 24,000 deaths. Many organisms have been investigated as potential agents for vector mosquito control, including viruses, fungi, bacteria, protozoa, nematodes, invertebrate predators and fish. However, most of these agents were shown to be of little operational use, largely because of the difficulty in

multiplying them in large quantities. *Bacillus thuringiensis* is seen as a biological control bacterium that presents several advantages over the use of chemical control agents, since the parasporal bodies released during their growth are highly specific for some of the major agronomical targets and with no effect on other non-target insects, plants and domestic animals. Six well characterized local isolates of *Bacillus thuringiensis* named as DAB-Btl-6 used to clone *cry10* gene were highly toxic to *Anopheles stephensi*. The 6-endotoxin gene *cry 10* was amplified by PCR sequenced and cloned in pTZ57 cloning vector. *cry 10* genes were cloned in pT7-7 expression vector and transformed in BL21 C for expression. Conditions were optimized to get good expression of *cry 10* gene. Bio toxicity assays against third instar of mosquito larvae *Anopheles stephensi* were done with genetically modified organisms and recombinant expressed Cry10 proteins. Bio toxicity assays showed the mosquitocidal activity of *cry 10* crystal protein gene.

MOLECULAR CHARACTERIZATION OF *cry11* GENE FROM LOCAL ISOLATE OF *BACILLUS THURINGIENSIS*

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Microbiological control of insects is considered as an important aspect of biological control and can be defined as the use of entomopathogenic microorganisms for insect control. Different species of *Bacillus* have been studied and special attention is given to *Bacillus thuringiensis* (*B.t*) and its various strains. At present *B.t* is one of the important and widely used bioinsecticides. In this study the optimal growth conditions (pH and temperature) of *Bacillus thuringiensis* was studied and different isolates of *Bacillus thuringiensis* were cultured at a pH range between 4-7 and a temperature range between 25-37°C. It was noticed that the neutral pH and 37°C temperature were most effective for their optimal growth. Growth curves for both local isolate (*SBS-B.t. 47*) and its positive control (HD500) were measured and compared them. To determine the percentile mortality and 50% lethal concentration, a range of concentrations (100/lg/ml - 1000/lg/ml) of spore diet was used in bioassays, for both local isolate as well as the positive control, against third instar of *Anopheles stephensi*. The optimal spore diet concentrations of local isolate and the positive control were 700/lg/ml and 900/lg/ml, respectively. A full length *crylla* gene of *B.t.* was released from an already cloned T/A vector (pTZ57R/T) and sub-cloned in pT7-7, an expression vector, under *EcoRI* and *HindIII* restriction sites. The construct was confirmed by restriction as well as sequence analysis in *Escherichia coli* (DH5a) subsequently transformed *Escherichia coli* BL21 C+ for expression. The expression was estimated by lowery method. The conditions for time and IPTG concentrations were optimized. A high level of expression was achieved at an IPTG concentration of 0.5mM and a temperature of 37°C after 3hrs.

BITTER GOURND YELLOW VEIN VIRUS- A FURTHER EVIDENCE FOR NATURAL RECOMBINATION AMONGST BEGOMOVIRUSES

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Momordica charantia (Cucurbitaceae), a vegetable crop commonly cultivated through Pakistan; and begomoviruses, a serious threat to crop plants, are natives of tropical and subtropical regions of the world. Leaf samples of *M. charantia* with yellow vein symptoms, typical of begomoviruses, and apparently healthy leaf samples were collected from areas around Lahore, Pakistan. Full-length clones of a bipartite begomovirus were isolated from symptomatic samples. The complete nucleotide sequences of the components of one isolate were determined and showed the arrangement of genes typical of Old World begomoviruses. The complete nucleotide sequence of DNA A showed 85.4% identity with an isolate of *Tomato Leaf Curl India Virus* (ToLCNDV) which showed that it is a distinct species of begomoviruses for which the name Bitter gourd yellow vein virus (BGYVV) is proposed. Molecular evidences showed that BGYVV emerged as a result of inter-specific recombination between isolates of ToLCNDV, and *Tomato leaf curl Bangladesh virus*. The complete nucleotide sequence of DNA B showed 86.4% identity with the Indian strain of *Squash Leaf Curl China Virus*.

GENE STUDY OF FIRST EXON IN WITHIN THE 5' FLANKING REGION OF GROWTH HORMONE GENE *BOS INDICUS*

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GH is a main regulatory protein secreted by pituitary gland and placenta, involved in most anabolic processes in mammals. Expression of more than one genes for GH has been reported, indicating polymorphism at gene and protein level, apart from this, silent mutations has also been reported related to the level of expression of GH gene. Aim of this study was to identify silent mutations within first exon in the 5' flanking regions of GH2 gene of *Bos indicus*. DNA was isolated from the blood of freshly slaughtered animal and a set of primer was used for gene amplification, binding at the region 241 +210 of GH gene at chromosome 19. DNA was amplified and the resultant product of about 453 bp was sequenced. The results showed that there were five single base silent mutations between TATA box for GH2 gene and the first exon, with three transversions and two transpositions, have not been reported before for GH2 gene allele in *B. indicus*.

These changes could be used as a genetic marker and perhaps related to the level of expression of GH2 gene.

**GENETIC DIVERSITY AND PREDATOR-PREY RELATIONSHIP IN SOME
SELECTED COLEOPTERAN AND HYMENOPTERANS SPECIES BY USING
RAPD MARKERS**

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Genetic diversity and predator- prey relationship of eleven insect species was studies by RPAD technique. Among them three Coleopteran species *Coccinella septumountata*, *Cheilomenes sexmaculata*, *Hippodemia convergens*, three Hymenopteran species *Camponotus pennsylvanicus*, *Solenopsis invicta*, *Formica rufa* and five Hemipteran species *Empoasca kerri*, *Macrosiphum miscanthi*, *Aphis maidis*, *Bemisia tabacii* and *Drosicha mangiferae* were accessed. A total of 175 fragments were amplified by using 25 RAPD primers. Out of which 159 fragments were polymorphic showing 91% polymorphism. The number of amplification products varied between 6 to 11 with an average of 7.00 per primer. Genetic characterization was done with the help of cluster analysis constructed on the basis of similarity matrix. Three main cluster groups were depicted. In one group only (carnivore) predator species were present alongwith a herbivorous prey *Empoasca kerri*. Second group contained three (herbivore) prey species while third group was solitary containing a single prey species *i.e.* *Drosicha mangifera*. A single band of approximately 850bp was identified in DNA sample of *Cheilomenes sexmaculata*. Similarly another band of approximately 700bp was identified in DNA sample of *Macrosiphum miscanthi*. These fragments could be used as fingerprints for the identification of *C. sexmaculata* and *M. miscanthi*. Predator-prey relationship was observed on the basis of comparison among control, fed predator and preys. Few unique fragments of aphid preys were identified in the fed coccinellid predators, suggesting a trophic link between predator and prey species studied.

GENETIC AND MOLECULAR ANALYSIS OF OCULAR ALBINISM

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Albinism, a complex group of genetic disorders characterized by defects in the synthesis or transport of melanin, resulting in reduced or complete absence of melanin

pigment in the skin, hair and eyes. Oculocutaneous Albinism (OCA) is an autosomal recessive disorder classified into several types based on clinical and molecular categories. OCA1 is caused by mutations in the tyrosinase gene (TYR 11q14-q21), OCA2, or tyrosinase positive OCA is caused by mutations in the p. protein gene (15q11.2-q12), OCA3, which is associated with mutations in the tyrosinase related protein gene (TYRP1 9p23), and OCA4 associated with mutations in the membrane associated transporter gene (MATP 5p). OCA1 can be further subdivided into two categories, OCA1A and OCA1B. OCA1A results from a complete lack of tyrosinase activity and produces a totally depigmented phenotype, while OCA1B or yellow albinism is characterized by a reduced rate of tyrosinase activity, and, as a result, individuals are born with white hairs that change to blond or yellow with age. Moreover, clinical overlap between the OCA forms is present, therefore, molecular and genetic analysis is necessary to establish the gene defect and OCA subtype. The purpose of this study was to carry out genetic and molecular analysis of oculocutaneous albinism in families from Multan, Pakistan as very little work had been done on families of this region. We collected five unrelated affected families from different areas of Pakistan. DNA from all the five affected families was extracted and emphasis was made on three families (PKAB002, PKAB005 and PKAB010) for molecular analysis due to lack of time. Samples of remaining families will be analyzed later and will further help in elucidation of melanogenesis and OCA in Pakistan. After extraction of DNA from three families (PKAB002, PKAB005 and PKAB010) four STR markers corresponding the each locus (OCA1, 2, 3, and 4) were amplified by PCR and analyzed on 6% denaturing PAGE. The results showed recessive pattern of inheritance, family PKAB002 and PKAB010 was found linked to known locus OCA type I (TYR) while family PKAB005 was found linked to OCA2 (P gene) locus of oculocutaneous albinism. This study has helped in molecular characterization of OCA in families PKAB002, PKAB010 and PKAB005. Further sequencing of the candidate genes will help in elucidating the effect of mutation on the functioning of the genes and protein.

DEGRADATION OF NITRO AROMATIC COMPOUNDS BY INDIGENOUS STRAINS OF BACTERIA

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Among the pollutants unique to the military and associated agencies are those arising from the manufacture, handling and demilitarization of munitions. 2, 4, 6-Trinitrotoluene (TNT) and hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine are widely used for military purposes. Disposal of the explosives during manufacturing and testing has resulted the contamination of water and soil ecosystems. Remediation of explosive contaminated soils and water and disposal of explosives are two important applications in which biological degradation of explosives may provide a safe and cost effective

approach. The research conducted in this project demonstrated that TNT can be removed effectively by native soil bacteria. Bacteria has shown best results of nitroaromatic compounds like TNT. Bacteria were grown on various media and it was observed that the media with glucose was most effective for the growth and degradation of TNT. Bacteria in order to check best degradation was exposed to temperature and it was found that 30°C is the best temperature for the growth and degradation. pH was also observed and it was concluded that in pH 7 bacteria degraded TNT most effectively. Nutrients like glucose were also a good experience in the determination of the bacteria to degrade TNT. Similarly Tween80 was found best for the degradation of the nitro aromatic compounds like TNT. It was observed during the experiment that bacteria when shifted from low concentration of nitro aromatic compounds towards the high concentration than bacteria can degrade TNT more than the 300 ppm. During the experiment bacterial strains *Nitrosomonas* and *Bacillus* degraded TNT of 300 ppm. Bacteria used TNT as the source of nitrogen and carbon. It was concluded that a consortium can be used for the biotreatment of TNT containing effluents.

PREVALENCE OF GLAUCOMA IN HOSPITAL POPULATION OF FAISALABAD

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Glaucoma is the second leading cause of blindness in the world and it is fourth leading cause of blindness in Pakistan. It causes blindness either in one eye or in both of the eyes. Present study was conducted to estimate the incidence, prevalence and risk factors responsible for glaucoma in local population. The data was collected from 234 patients of glaucoma in different hospitals and private clinics of Faisalabad. Data was collected with the help of a questionnaire and it was statistically analyzed for percentage (%), average, chi-square test and coefficient of inbreeding (F). Out of 234 patients of glaucoma, 130 were males and 104 were females. Twelve types of glaucoma patients were encountered during the study. Primary open-angle (41.88%), primary angle closure (19.23%) and childhood (9.40%) glaucoma were more common types while lens-induced glaucoma (SACG and SOAG) were less common types (0.85% and 0.43%, respectively). Average age of diagnosis revealed that glaucoma had late onset. Average age of diagnosis was 52.59 ± 1.44 years and average age at present was 53.64 ± 1.43 years. Age group 55-59 years was more prone to the disease (14.10%). Most of the patients were married (79.06%). The highest representation of all types of glaucoma patients was in 3rd birth order (21.79%) and lowest in 9th birth order (0.85%). Maximum percentage of male patients was observed in 3rd birth order (25.38%) and among females, 1st birth order was more common (20.19%). Risk factors including intra ocular pressure (IOP), Diabetes and hypertension was also observed. Mean (IOP) of glaucoma patients was found to be 28.12 ± 0.38 mm Hg. Lowest IOP (13.57 ± 0.20 mm Hg). Prevalence of Diabetes (22.22%)

and hypertension (43.16%) was observed among glaucoma patients among blind glaucoma patient (14.10%), 60.61% patients were unilaterally blind while 39.39% patients were bilaterally blind. Blindness was more common among females (51.52%) than males (48.48%). Unilaterally blindness was common in primary open-angle glaucoma (76.47%) while in primary angle closure (71.43%) and childhood glaucoma (100%), bilateral blindness was more common.

EFFECT OF COW DUNG ON BIOASSIMILATION OF LEAD AND CADMIUM IN IN EARTHWORM (*PHERETIMA POSTHUMA*)

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The Present study was designed to investigate the effect of cow dung on Bioassimilation of lead and cadmium in earthworm (*Pheretima posthuma*). Samples of earthworm were analysed by the Atomic absorption spectrometer for two heavy metals viz. Pb and Cd. Bioassimilation of Pb and Cd by earthworm was found statistically highest in CD^a (Initial substrate that contains 245gram soil and 5gram cow dung). The content of both metals were statistically significantly lower in casting than initial substrate and control. Positive effect of cow dung was found.

THE EFFECT OF FENVALERATE ON THE DEVELOPMENT OF CHICK

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The main objective of the present study was to test the effects of fenvalerate (Fen) in chick embryo, which is a synthetic pyrethroid, and commonly used for destroying a variety of insect pests damaging several vegetables, fruits and cotton crop. Human beings are exposed to formulated fenvalerate preparations, mostly by inhalation during spray in the fields for crop protection, to mitigate household insects and also during handling and picking. Fresh eggs (Lyalpur Silver Black Breed) were purchased and incubated at 37±0.5°C. After seven days of incubation, eggs were divided into two segments. Each segment consisted of 24 eggs. In the single segment one group (eight eggs) remained as control group while other two groups were treated with 0.1 and 0.05 µg of fenvalerate/ml/egg with the help of micro applicator. Same procedure was adopted for the second segment. Recoveries were made on 14th and 20th of incubation and embryos were preserved in 80 % alcohol. Observations involved measurement of weight crown rump length, beak length and development of eyes. Observations revealed concentrations dependant adverse effects of insecticide. The developmental defects were loss in weight,

reduction in crown rump and beak length whereas the eyes were normal. The present study indicated that Fenvalerate is potentially dangerous avian development even at very low dose concentration. This study suggested that this insecticide must be used with utmost consideration and care.

EFFECT OF 17 α -METHYLTESTOSTERONE ON MASCULINIZATION, GROWTH PERFORMANCE AND MEAT QUALITY IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) AT DIFFERENT DIETARY PROTEIN LEVELS

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The main objective of the present study was to investigate the effect of androgen hormone 17 methyltestosterone (MT) and dietary protein on masculinization, growth performance and meat quality in Nile tilapia (*Oreochromis niloticus*). Sexually undeveloped tilapia fry were orally administrated with 60 and 70 mg MT/Kg of feed @ 30 and 40% dietary crude protein levels for 30 days in glass aquaria. After this fry were shifted to earthen ponds to monitor its growth performance and it lasts for 180 days. At the final harvest frequency data of males and females was determined by microscopic examination of gonads and morphometric characteristics in terms of wet body weight and total length was recorded to determine the growth performance. Study results showed that masculinization and growth performance increased in tilapia with increasing hormone doses of 17 α -MT and dietary crude protein. Highest percentage of males (93-100%) was obtained @ the dose rate of 70mg MT/kg with 30 and 40% dietary crude protein levels. Fish showed highest growth performance (final weight, weight gain and growth rate) @ 40% crude protein diet with 70mg MT/Kg. Protein levels and hormone doses significantly affect the meat quality in terms of protein, lipid, fibre and ash contents. Protein and ash contents of fish body was significantly increase ($P < 0.05$) with increasing protein and hormone doses in the diet while lipid and fibre contents showed an inverse relationship with increasing trend. Most of the ecological parameters of pond water showed highly significant seasonal differences but remained favourable during whole period of study. In conclusion, by increasing hormone doses (MT) and dietary protein levels masculinization and growth rate can be increased remarkably in Nile tilapia.

THE EFFECT OF ENDOSULFAN ON THE DEVELOPMENT OF CHICK

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The main objective of the present study was to test the effects of endosulfan in chick embryo, which is a highly toxic pesticide and is commonly used to destroy a

variety of pests of vegetables, fruits and crops. Fertilized fresh eggs (Lyallpur Silver Breed) were purchased and incubated at $37\pm 1^{\circ}\text{C}$. After seven days of incubation, eggs were divided into two sets composed of three groups each for experiment. In the first set, the experiment was run up to 14th day of incubation in which two groups were treated with different concentrations of endosulfan (0.1 and 0.05 $\mu\text{g/ml/egg}$) and one group was kept untreated as control. In the second set of experiment that spans for 20th day of incubation in which two groups were treated with different concentrations of endosulfan (0.1 and 0.05 $\mu\text{g/ml/egg}$) and one group was kept untreated as control. Required concentrations were injected in the albumin with the help of micro applicator. Observations revealed concentrations dependant adverse effects of the pesticide. The developmental defects were reduction in crown rump, loss in weight and short beak but no abnormalities were observed in eyes. The present study indicated that endosulfan is potentially dangerous to avian development even at very low concentration. This study suggested that this pesticide must be used with utmost care and according to well thought plans because it may be equally harmful to mammals especially human development.

EXTRACELLULAR ENZYME PRODUCTION BY INDIGENOUS THERMOPHILIC BACTERIA ISOLATED FROM HOT SPRINGS

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The applications of thermophilic microorganisms in industrial processes have opened a new era in biotechnology. This group has unique features, which can be exploited for use in biotechnological and socio-economic industries. They have shown tremendous potential because of their ability to produce unique bioactive molecules that are also thermostable. The study is aimed to decipher indigenous resources and isolate microbial gems. In this regard about 200 bacteria were isolated from Manghopir hot springs. Among these isolates gram positive bacteria were dominant over gram negative bacteria and both thermophilic and thermotolerant group of bacteria were identified. These bacteria were screened for their potential for amylase, β -galactosidase, cellulase and protease using both spectrophotometric and plate assays. Different combinations of enzyme production were observed among different isolates. However, some isolates were found to secrete all of the aforementioned enzymes. Studies on α -amylase were carried out with a bacterial strain (identified as *Bacillus* sp.) on the basis of maximum starch hydrolysis. Optimization of the enzyme production and characterization of the partially purified enzyme was performed. The high temperature and pH stability profile revealed that the *Bacillus* sp. may be regarded as a promising source of α -amylase for biotechnological and industrial applications.

COMPARATIVE EFFECTS OF BIOSAL AND PERMETHRIN ON ACTIVITY OF ALKALINE PHOSPHATASE (ALP) ENZYME IN BRAIN, HEART, LIVER, KIDNEYS, GILLS AND MUSCLES OF *CYPRINUS CARPIO*

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Usage of pesticides has paradoxical impacts *i.e.*, on one hand these pesticides enhance the quantity of food production while on the other hand these are posing a serious threat to the environment. In this experimental work *Cyprinus carpio* (common carp) was exposed to 24 hours LC_{50} of permethrin and Biosal (Neem based phytopesticides). Alkaline phosphatase (ALP) activity was calculated in various fish organs (brain, heart, liver, kidneys, gills and muscles) of Biosal and permethrin treated as well as untreated (control) fish samples. In case of Biosal treated fish ALP activity was noted to inhibit in all the organs (-44% in kidneys, -38% in heart, -34% in muscles, -32% in liver and -21% in brain) except gills in which ALP activity was elevated (+35%). In permethrin treated *Cyprinus carpio*, inhibition in ALP activity was also noticed in all the tested organs (-73% in muscles, -60% in heart, -57% in brain, -51% in kidneys and -48% in liver) with the exception of gills (+25% elevation in ALP activity). Inhibition in ALP activity caused by Biosal and permethrin was statistically significant in brain, liver, kidneys and muscles while insignificant elevation was observed in gills.

OCCURRENCE OF *SALMONELLA* SPP. IN BLACK RAT (*RATTUS RATTUS*), HOUSE MOUSE (*MUS MUSCULUS*) AND MONGOOES (*HERPESTES JAVANICUS*) AT POULTRY FARMS OF RAWALPINDI/ISLAMABAD, PAKISTAN

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This study was designed to determine the prevalence of *Salmonella* spp. in black rat (*Rattus rattus*), house mouse (*Mus musculus*) and mongooses (*Herpestes javanicus*) residing at poultry farms in Rawalpindi/Islamabad. For this purpose, fifty three animals (black rat, 32, house mouse, 16; mongoose, 05) were captured to check the presence of

Salmonella spp. in faecal matter, urine and blood. Prevalence of *Salmonella* spp. was 56% and 28% in fecal matter and urine of black rat, respectively. In house mouse, prevalence of *Salmonella* spp. was 13% in fecal matter. However, entire captured mongoose were negative for *Salmonella* presence. In conclusion, black rat and house mouse are the main reservoirs for *Salmonella* spp. at poultry farms of Rawalpindi/Islamabad.

PREVALENCE OF BOVINE TUBERCULOSIS IN CATTLE AND BUFFALO IN ISLAMABAD, PAKISTAN

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Bovine tuberculosis (BTB) causes significant economic loss to agricultural communities and is public health hazard in several countries. An attempt was made to investigate the prevalence of bovine tuberculosis (BTB) in cattle and buffalo in Islamabad area, Pakistan. For this purpose, a total of 200 cattle and 200 buffalo from 10 randomly selected farms, were screened by using single comparative intradermal tuberculin test (SCITT). Milk samples from all tuberculin positive and negative animals giving mild reaction to tuberculin were cultured for bacteriological examination. Farm workers were screened for tuberculosis using Mantoux test. To see the effect of tuberculin administration on milk yield of animals, daily milk yield from 12 buffaloes were noted 15 days before and 15 days after tuberculin administration. The information about effect of age, sex, milk yield, breed, herd size, body mass and parturitions on prevalence of tuberculosis and awareness of farm workers/owners about disease were collected through questionnaire. Prevalence of BTB was found to be 1.5% in cattle and 2.0% in buffalo. Milk of positive animals was found free from bacteria. All the farm workers were found healthy after screening. Drop in milk yield was noted after 12 hours of tuberculin administration in two buffaloes. Older age, poor hygienic condition and large number of animals living together favoured the prevalence of disease. Knowledge of workers/owners about the disease was poor. It was concluded that prevalence of tuberculosis in dairy animals in Islamabad area was low (1-2%) and most of the herds were BTB free. It was felt that owners of the animals and workers at the dairy farms needed to get education about zoonotic diseases.

DETERMINATION OF HEAVY METALS IN THE WATER AND FISHES OF GALPANI NALLAH (MARDAN)

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The present study has been designed to determine the level of Heavy metals in water and fishes of Galpani Nullah, Mardan, NWFP. The levels of heavy metals (Pb, Cd, Zn, Cu, Ni and As) were measured in water samples varied in different months *i.e.* lead 0.823 – 1.647 mg/l, cadmium 0.550 – 1.173 mg/l, zinc 0.653 – 1.563 mg/l, copper 0.447– 0.937 mg/l, nickel 0.370– 1.140 mg/l and arsenic 0.177 – 0.510 mg/l. Total 8 fish species were collected and identified *i.e.* *Cirrhinus reba* (Bhangan), *Puntius ticto* (Chudoo), *Oreochromis niloticus* (Tilapia), *Cyprinus carpio* (Gulfam), *Labeo dyochelis* (Torkey), *Puntius sarana* (Olive barb), *Clupisoma naziri* (Shermahi) and *Vallagu attu* (Mallee). The levels of heavy metals (Pb, Cd, Zn, Cu, Ni and As) were measured in different tissues (muscles, gills heart, scales, kidney and liver) of fish species *i.e.* Average Pb contents in different tissues varied from 12.76±0.24 to 41.03±4233 µg/g in *Cirrhinus reba* (Bhangan), 17.07±0.63 to 45.18±0.56 µg/g in *Puntius ticto* (Chudoo), 9.20±0.25 to 38.50±0.55 µg/g in *Oreochromis niloticus* (Tilapia), 12.10±0.16 to 20.97±0.14 µg/g in *Cyprinus carpio* (Gulfam), 11.67±0.36 to 17.13±0.15 µg/g in *Puntius sarana* (Olive barb), 9.60±0.23 to 18.60±0.16 µg/g in *Wallagu attu* (Mallee), 16.10±0.45 to 47.60±0.57 µg/g in *Clupisoma naziri* (Shermahi) and 18.33±0.31 to 45.67±0.26 µg/g in *Labeo dyochelis* (Torkey). Average Cd contents in different tissues varied from 17.87±0.32 to 25.97±0.32 µg/g in *Cirrhinus reba* (Bhangan), 14.79±0.38 to 26.53±0.45 µg/g in *Puntius ticto* (Chudoo), 12.97±0.27 to 20.27±0.23 µg/g in *Oreochromis niloticus* (Tilapia), 13.40±0.13 to 19.17±0.13 µg/g in *Cyprinus carpio* (Gulfam), 8.43±0.13 to 17.63±0.11 µg/g in *Puntius sarana* (Olive barb), 9.23±0.15 to 17±0.13 µg/g in *Wallagu attu* (Mallee), 20.87±0.49 to 34.27±0.20 µg/g in *Clupisoma naziri* (Shermahi) and 23.63±0.40 to 40.50±0.45 µg/g in *Labeo dyochelis* (Torkey). Average Zn contents in different tissues varied from 95.5±0.17 to 137.97±0.42 µg/g in *Cirrhinus reba* (Bhangan), 89.97±0.21 to 136.53±0.42 µg/g in *Puntius ticto* (Chudoo), 95.83±0.42 to 144.77±0.27 µg/g in *Oreochromis niloticus* (Tilapia), 72.80±0.17 to 93.73±0.23 µg/g in *Cyprinus carpio* (Gulfam), 76.70±0.12 to 121.60±0.49 µg/g in *Puntius sarana* (Olive barb), 94.47±0.13 to 124.03±0.13 µg/g in *Wallagu attu* (Mallee), 123.73±0.33 to 153.77±0.47 µg/g in *Clupisoma naziri* (Shermahi) and 117.13±0.27 to 168.57±0.37 µg/g in *Labeo dyochelis* (Torkey). Average Cu contents in different tissues varied from 107.40±0.13 to 148.17±0.57 µg/g in *Cirrhinus reba* (Bhangan), 107.40±0.24 to 138.97±0.38 µg/g in *Puntius ticto* (Chudoo), 98.80±0.19 to 125.63±0.22 µg/g in *Oreochromis niloticus* (Tilapia), 98.90±0.19 to 125.67±0.19 µg/g in *Cyprinus carpio* (Gulfam), 89.50±0.13 to 125.37±0.38 µg/g in *Puntius sarana* (Olive barb), 108.53±0.28 to 128.77±0.67 µg/g in *Wallagu attu* (Mallee), 117.13±0.39 to 156.20±0.38 µg/g in

Clupisoma naziri (Shermahi) and 124.40 ± 0.57 to 143.47 ± 0.59 $\mu\text{g/g}$ in *Labeo dyochelis* (Torkey). Average Ni contents in different tissues varied from 27.03 ± 0.65 to 108.17 ± 0.57 $\mu\text{g/g}$ in *Cirrhinus reba* (Bhangan), 18.30 ± 0.14 to 95.27 ± 0.15 $\mu\text{g/g}$ in *Puntius ticto* (Chudoo) 8.53 ± 0.56 to 79.93 ± 0.18 $\mu\text{g/g}$ in *Oreochromis niloticus* (Tilapia), 61.73 ± 0.57 to 117.80 ± 0.57 $\mu\text{g/g}$ in *Cyprinus carpio* (Gulfam), 75.83 ± 0.18 to 126.27 ± 0.47 $\mu\text{g/g}$ in *Puntius sarana* (Olive barb), 48.23 ± 0.18 to 73.40 ± 0.35 $\mu\text{g/g}$ in *Wallagu attu* (Mallee) 36.97 ± 0.26 to 126.67 ± 0.42 $\mu\text{g/g}$ in *Clupisoma naziri* (Shermahi) and 67.33 ± 0.33 to 131.03 ± 0.42 $\mu\text{g/g}$ in *Labeo dyochelis* (Torkey). Average As contents in different tissues varied from 36.17 ± 0.10 to 53.97 ± 0.42 $\mu\text{g/g}$ in *Cirrhinus reba* (Bhangan), 34.77 ± 0.16 to 56.93 ± 0.49 $\mu\text{g/g}$ in *Puntius ticto* (Chudoo) 38.23 ± 0.11 to 56.70 ± 0.49 $\mu\text{g/g}$ in *Oreochromis niloticus* (Tilapia), 39.34 ± 0.23 to 53.44 ± 0.29 $\mu\text{g/g}$ in *Cyprinus carpio* (Gulfam), 40.66 ± 0.37 to 55.75 ± 0.41 $\mu\text{g/g}$ in *Puntius sarana* (Olive barb), 42.18 ± 0.45 to 59.13 ± 0.27 $\mu\text{g/g}$ in *Wallagu attu* (Mallee) 41.47 ± 0.42 to 56.98 ± 0.19 $\mu\text{g/g}$ in *Clupisoma naziri* (Shermahi) and 42.23 ± 0.17 to 64.67 ± 0.48 $\mu\text{g/g}$ in *Labeo dyochelis* (Torkey).

IDENTIFICATION AND ANTIBIOTIC RESISTANCE PROFILE OF BACTERIAL ISOLATES FROM CASES OF SORE THROAT IN CHILDREN

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Respiratory infections are amongst the most wide spread and serious infections, accounting for over 50 million deaths globally each year. This study was designed to isolate and identify the bacterial strains from cases of sore throat in children (age < 15 years). and to check their resistance profile against commonly used antibiotics. The antibacterial activity of some natural products like vegetable oils and honeys was also investigated against these bacterial strains. Clinical samples (throat swabs) were collected from 30 patients, three cases were found to be negative and total 45 strains were isolated from 27 cases. Bacterial isolates were subjected to a series of microbiological and biochemical tests for identification. Among these *S. aureus* was found to be the most common (82.2%), followed by β -hemolytic *Streptococci* Spp. (17.7%). These isolates were subjected to culture sensitivity test against fourteen known antibiotics. Chloramphenicol, Rifampicin, Vancomycin, Levofloxacin, Gentamycin and Cefixime were found to be effective for all strains of both *S. aureus* and β -hemolytic *Streptococci* spp., on the other hand all isolates were resistant to Oxacillin, Ceftriaxone and Amoxicillin. Variable level of resistance was recorded against Clarithromycin, Erythromycin, Tetracycline and Bacitracin. The antibacterial potential of nine vegetable oils and three different types of honeys was also worked out for selected resistant isolates. Among oils, cardamom oil, black seed oil and clove oil displayed antimicrobial potential on *S. aureus* and β -hemolytic *Streptococci* Spp., while all three honeys, garlic, berry and natural/commercial honeys also showed variable antibacterial activity.

**AMINO ACIDS PRODUCTION BY SOME LOCALLY ISOLATED BACTERIA
IN CORN STEEP LIQUOR (CSL) MEDIUM**

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Strains of amino acid(s) producing bacteria were isolated from two different sources *viz.* water and soil, and their production potential was checked in Corn Steep Liquor medium. Qualitative and quantitative analysis of amino acids was performed by paper chromatography and Spectrophotometry. Amino acid production ranged from 0.1-6.7 mg/l in broth culture in 72 hours of incubation under optimized conditions of Temperature (37C), pH and inoculum size. Total frequency of strains studied in the medium understudy was thirty and the strain frequencies for, Lysine, Aspartic acid, Phenylalanine, Proline, Methionine, Isolucine, Valine, Alanine, Glutamic acid and Histidine production were 19, 19, 20, 20, 20, 22, 23, 25, 25 and 27 frequencies respectively.

**COMMON SHREW (*SUNCUS MURINUS*): A POTENTIAL RESERVOIR OF
PATHOGENIC BACTERIA AT POULTRY FARMS, RAWALPINDI, PAKISTAN**

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The prevalence of pathogenic bacteria was checked in the fecal matter, urine and blood of common shrew (*Suncus murinus*) inhabiting poultry farms. Thirteen shrews were captured and checked for presence of pathogenic bacteria from different poultry farms of Rawalpindi/Islamabad. Prevalence of *Escherichia coli* and *Proteus* spp. were 62% and 13% in fecal matter, respectively. Prevalence of *Salmonella* spp. was 69% in fecal matter and urine. It is concluded that common shrews serve as reservoir of pathogenic bacteria, and can be prevented by adopting suitable strategies to eliminate the shrew populations from the poultry farms.

IDENTIFICATION OF BACTERIAL ISOLATES FROM CONTACT LENSES/LENS CASES AND ANTIMICROBIAL POTENTIAL OF SOME VEGETABLE OILS AGAINST SELECTED RESISTANT STRAINS

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Contact lenses are used for therapeutic as well as cosmetic purposes; the usage of contact lenses may give rise to problems like eye infections, allergic reactions to lens or lens care solutions, scratched corneas, alteration in corneal shape and abnormal blood vessels growing in the apparently clear cornea. The present study was conducted to characterize the bacterial isolates from contact lenses and lens cases and their responses to some natural products. A total of 42 samples of lenses/lens cases were collected from the regular contact lens users. Out of these 40 bacterial isolates, 16 gram positive and 24 gram negative were obtained. The bacterial isolates included *Staphylococcus epidermidis*, *Klebsiella* Sp., *E.coli*, *Citrobacter* Sp. and *Pseudomonas auruginosa*. Most of the isolates were found to be resistant to more than three commercial antibiotics. Ten different vegetable oils were used to check the antibacterial activity against selected isolates. Three oils obtained from clove, cardamom and black seed were found to be effective in restricting the growth of these pathogens.

IMMUNE RESPONSE IN CHICKEN BY OIL BASED VACCINE PRODUCED BY LOCAL ISOLATES OF MYCOPLASMA GALLISEPTICUM

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Mycoplasma gallisepticum (MG) is the cause of Chronic Respiratory Disease (CRD) in poultry; imported vaccine is costly and its availability is difficult. The aim of the study was to prepare a killed vaccine of the local isolates of MG. Organism was isolated from breeder farms located at Tret, Tarli and Sehala area of Islamabad. Isolates were identified and grown on PPLO broth. EID 70 of the cultures was calculated and antigen was inactivated by 0.1% Formaline, washed three times in sterile PBS. Final formulation of vaccine was prepared by mixing with montanide oil based with the ratio of 40:60. Antibody titer was detected with the ELISA Kit. Three vaccines A, B, and C, were prepared from three different isolates and broilers of 14 days were maintained for the experiment. Three groups for 10 birds for each were vaccinated and a group of 10 birds

was kept as control. Antibodies titer was 4 and 7 of 15 and 30 days of postinoculation. Birds were challenged with the EID 70 dose of respective antigen where as three birds of control group were also inoculated by challenging dose and 1 bird was kept as negative control. The vaccine from local isolates was found effective. Based on this study oil based vaccine can be used against CRD but a large scale study is to be needed in this respect.

FREQUENCY DISTRIBUTION OF ABO BLOOD GROUPING AMONG THE THALASSEMIC PATIENT IN NAWAB SHAH (DISTT: SHAHEED BENAZIR ABAD) AND HYDER ABAD, SINDH

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This study was carried out for the presence of ABO and Rh(D) Blood groups in the thalassemic patients. Out of total 442, the percentage distribution of A, B, AB and O were 24.88%, 27.14%, 7.69% and 40.27% respectively. Frequencies of A, B and O alleles were 0.172 ± 0.017 , 0.187 ± 0.017 and 0.634 ± 0.021 respectively. Among all of these 93.39% were Rh+ve with frequency of 0.745 ± 0.011 and Rh-ve were 6.1% with frequency of 0.255 ± 0.073 , while another 214 patients of the thalassemia were collected at Hilal-e-Ahmer Fatimed Foundation Hyder abad. Out of total 214, the percentage distribution of A, B, AB and O were 18.69%, 36.91%, 8.41% and 35.98% respectively. Frequencies of A, B and O alleles were 0.139 ± 0.020 , 0.254 ± 0.025 and 0.599 ± 0.035 respectively. Among all of these 89.25% were Rh +ve with frequency of 0.653 ± 0.021 and Rh -ve were 10.75% with frequency of 0.347 ± 0.084 . This cross sectional study was performed at thalassemia care centre district Shaheed Banazir abad Sindh Pakistan, between February 2007 to September 2009. Thalassemia care centre (T.C.C) is only referral centre for the diagnosis and treatment of thalassemia in the mid of Sindh, while Hilal-elAhmer Fatimed Foundation Southern sindh Hyder abad. All thalassemic patients from T.C.C and H.F.F, who received regular blood transfusion, were included in this study. Blood groups of patients with thalassemia major were performed after, they clinically diagnosed and confirmed on laboratory based tests. Blood grouping of 442 patients from Shaheed Benazir abad and 214 patients from Hyder abad of the thalassemia were performed by technique described by the Karl Landstiener and Wiener. Blood groups were checked by slide method by using biological Inc. The Performa was designed to record ABO and Rh-D as well as age and sex of thalassemic patients. The results were analyzed statistically by using Harday-Weinburg Law.

COMPARATIVE CLINICAL EFFICACY OF TWO PLANTS (*FUMARIA PARVIFLORA*, *ARTIMISIA VELGARIS*) AGAINST TOXAEMIA IN BUFFALOES

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Two self growing plants (*Fumaria parviflora* (Shatrah) and *Artimisia velgaris*(Afsanteen)in Punjab province were tried against toxaemia (Zaharbad) in buffaloes(n=45) under field conditions. Fresh plants were cut and chopped to dry in shade for 15 days followed by pulverization. Forty five buffaloes were randomly divided into 3 equal groups viz A to C. Each group was further divided into 3 sub groups (mild, moderate and severe) on the basis of skin discolouration and ocular mucopurulent discharge scoring it as mild(+), moderate(++) and severe(+++). Shatrah, Afsanteen and their blend (1:1) were given to the animals of group A, B and C respectively @ 100gm/animal daily for 7 consecutive days followed by their monitoring till complete recovery. In all groups mild, moderate and severely affected animals were recovered in 3, 4 and 5 days. As an average on group basis clinical symptoms disappeared in 4, 5 and 2 days in groups A, B and C, respectively. After recovery, the skin of all animals became pitch dark and eyes became bright besides increased production and feed intake. It was concluded that these two plants can be used as antitoxaemic agents individually without having any side effects but their blend is an excellent one and cheap, inexpensive method to ameliorate the toxaemia in buffaloes.

COMPARATIVE EFFICACY OF *ARTMISIA VELGARIS* (AFSANTEEN) AND *NIGELLA SATIVA* (KALVANGI) FOR THE TREATMENT OF RETAINED PLACENTA IN BUFFALOES

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Pulve afsanteen and kalvangi were tried on 30 buffaloes affected with retained placenta during 02 years period (2008 to 2010) under field conditions. These animal were divided into 03 equal groups i.e. A through C. Pulve afsanteen, *Nigella sativa* and their blend were given to the animals of groups, A, B and C respectively @ rate of 100gms /animal *per os* twice a day. In group A, 7 animals threw their placenta with in 24 hours and 8 animals from group B threw their placenta with in 24 hours, while all animals of group C threw the placenta with in 12 hours after the medication. On the other hand, the remaining animals of groups A and B thrown it till 36 hours. It was concluded that afsanteen and kalvangi can be used as ecbolics but their blend quickens the uterine contraction to remove placenta with in short time. It is also a cheap method to get this problem resolved.

HYPERHOMOCYSTEINEMIA AND B-VITAMIN DEFICIENCIES IN A PAKISTANI POPULATION IN KARACHI

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Hyperhomocysteinemia is a risk factor for atherosclerosis. The objective of this study was to find out the prevalence of hyperhomocysteinemia, and deficiencies of folate, vitamin B6 and vitamin B12 in an urban population in Karachi, Pakistan. A cross-sectional study followed by intervention in a subgroup was carried out in a low income population in Karachi recruiting 872 adults aged 18-60 years. Fasting venous blood was obtained. Serum was analyzed for folate and vitamin B12. Plasma was analyzed for pyridoxal phosphate (PLP, coenzymic form of B6) and total homocysteine. A group of vitamin-deficient individuals (n=194) was given 3-week supplementation with folic acid, methycobalamin and pyridoxine hydrochloride. After supplementation, serum/plasma levels of folate, vitamin B12, PLP and homocysteine were again determined. Prevalence of hyperhomocysteinemia ($>15\mu\text{mol/l}$) was 32%. Similarly percent values of folate deficiency ($\leq 3.5\text{ng/ml}$), vitamin B6 deficiency ($\text{PLP} \leq 20\text{ nmol/l}$) and vitamin B12 deficiency ($\leq 200\text{pg/ml}$) in the study population were 27.5%, 33.7% and 9.74%, respectively. Hyperhomocysteinemia was associated with male sex, folate deficiency, vitamin B12 deficiency [OR(95%CI), 8.3(5.7-12.1); 2.5(1.76-3.58); 2.6(1.5-4.5), respectively]. Tobacco chewing was also found to be associated with hyperhomocysteinemia ($p<0.001$). A 3-week supplementation with folic acid (5mg/day), methycobalamin (0.5mg/day) and pyridoxine hydrochloride (vitamin B6, 50mg/day) in vitamin-deficient subjects (n=194) decreased plasma homocysteine levels by 37%. High prevalence of folate, vitamin B12, and vitamin B6 deficiencies appear to be the major determinants of hyperhomocysteinemia in a low income general population in Karachi

ASTHMA AND ALLERGIES FROM FUNGAL BIO-AEROSOLS IN THE ATMOSPHERE OF KARACHI

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The nature is highly diverse and fungal spores are among the most common bio-aerosols that human inhale from air. Fungus is known to be one of the causative allergenic agents that aggravate allergic rhinitis, bronchial asthma and allergies that also

due to house dust mites, pollen and pet dander. Outdoor airborne fungi play important role in IgE antibody mediated hypersensitivity. Interest in measuring exposure to airborne fungi has increased dramatically in recent years due to the complex and diverse associations suspected to occur between such exposure and adverse health effects. Hence, this rather small group of fungi has the potential of affecting the health of millions of people worldwide, ultimately resulting in enormous economic losses in the form of agricultural yield and medical care costs. To our knowledge there are no available data of whole year with hourly variation in Pakistan on the atmospheric concentration of fungal spores. Therefore, aerobiological studies are first time performed using Burkard Volumetric 7-Days Recording Sampler to determine airborne biological particles like fungal spores and pollens. From the preliminary data during the period of January–December 2007, 60 fungal genera were identified. The quantity and diversity of the fungi vary from location to location depending upon the environmental factors like temperature, humidity, heat index, dew point, wind velocity, rain, and bar pressure. Fungal spores can never be completely removed from the air and surrounding environment. Therefore, patients of respiratory and skin allergies need to be advised to avoid exposure to fungi and take necessary safety measures in those months in which fungal spores are expected to be higher in concentration. However, environmental factors and allergenic fungal spore calendar will help the physicians and patient for disease management.

A NEW NON-GENETIC NON-INSULIN DEPENDENT TYPE 2 DIABETIC RAT MODEL

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There are many animal models representing and claiming as non insulin dependent type 2 diabetic models (NIDDM) but none of them is given a status of a true model. That is why, different animal models are being represented until today and efforts to develop more realistic model is still under the way. The purpose of this study was to develop a new non-genetic NIDDM rat model that closely simulate the progression of pre-diabetes and the metabolic abnormalities of both pre and post diabetic stages to that of human disease. In this way, male Sprague Dawley (SD) rats were fed standardized high fat diet (HFD) with known composition of saturated/unsaturated fatty acids as well as the proportion of each fatty acid and periodically determined the metabolic changes occurred. The metabolic state was determined via Oral fasting glucose tolerance test, insulin sensitivity index, C-peptide and glucose transporter (Glut 4) expression etc. HFD was continued through insulin resistance stage (high insulin / normoglycemic) till the progression of pre-diabetic stage (high insulin / hyperglycemic). Following that, a low dose streptozotocin (STZ) (30 mg/kg) was injected to covert this pre-diabetic stage into

the diabetic stage (low insulin / hyperglycemia). A true insulin resistance stage was developed three months post HFD initiation and it takes six months to become a pre-diabetic stage. Another month was spent to develop a NIDDM diabetic model that is one month post STZ injection. Conclusively, in comparison to short term NIDDM animal models which skip certain intermediary stages, this model progresses through all declared human pre-diabetic stages till the metabolic states of NIDDM.

SCREENING OF FUNGUS CAPABLE OF LACCASE PRODUCTION IN DIFFERENT CULTURE MEDIAS

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Laccase is a copper containing polyphenol oxidase that act on wide range of substrates. This enzyme is found in many plant species and is extensively distributed in fungi including white rot fungi. Fungus capable of Laccase (EC 1.10.3.2; benzendiol:oxygen oxido-reductase) production was evaluated by plate test. Oxidation of guaiacol was evaluated in order to gauge the prospective of different strains of fungus to augment laccase production by basidiomycetes. Out of six cultures tested only two cultures were found laccase positive with *Coriolous Versicolorus* and *Pleurotus Ostreatus* being the most excellent potential culture. Laccase production was studied on six diverse liquid Medias (09-CBZOOI-006) by two different fungi. 09CBZ001 was found best media because of maximum activity 121 U/ml and 118U/ml in *Coriolous Versicolorus* and *Pleurotus Ostreatus* respectively in comparison with all other medias.

EFFECT OF OBESTATIN ON PLASMA TESTOSTERONE AND PROLACTIN SECRETION IN MALE SPRAGUE DAWLEY RATS

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The present study was designed to investigate in vivo effect of an anorexigenic gut peptide obestatin on plasma testosterone and prolactin concentration in adult male Sprague Dawley rats. The animals were anesthetized with diethyl ether. Sequential blood sampling was carried out through a teflon cannula implanted in the lateral tail vein. One group of animals served as control while second group was injected with single dose of 8nmol/Kg of obestatin. Blood samples (200/11-250 /11) were obtained at -10, 0, 10, 20, 30 and 40 minutes after treatment at 0 minutes. Hormonal concentrations were determined using Enzyme Immuno Assay kits. In treated group obestatin caused a

significant ($p < 0.05$) increase in plasma testosterone concentrations at 20 minutes. A non significant ($p > 0.05$) increase was noticed at 30 and 40 minutes after treatment. A significant ($p < 0.05$) increase in plasma testosterone concentrations was observed between mean pre treated and mean post treated samples. Obestatin showed no profound effect on plasma prolactin concentration in all the treated groups. In conclusion the present study suggests that this new gut peptide may be involved in regulation of testosterone secretion however it has no effect on the prolactin secretion in adult male rats. A detailed study is required to confirm the role of this anorexigenic gut peptide in controlling hypothalamic pituitary gonadal axis.

DETERMINATION OF HEAVY METALS (Cd, Cr, Co, Cu) IN THE *CIRRHINA MRIGALA* FISH TISSUES

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The discharge of untreated industrial wastes and domestic sewage, coming from various industries, make their passage to the River Ravi, Pakistan. This study was planned to assess the concentration of various toxic metals viz. Cd, Cr, Co and Cu in *Cirrhina mrigala* captured from Baloki Head works. Eight sampling stations were fixed to assess the river water quality and tributary pollution in the light of physico-chemical parameters, viz. water temperature, pH, dissolved oxygen, total hardness and metal ion viz. Cd, Cr, Co and Cu. The results showed significant differences among six fish organs viz. Gills, kidney, liver, skin, muscle and scales. Accumulation of Cu in all the six fish organs showed significant difference. Fish liver was the organ that accumulated significantly higher quantities of these metals followed by that of kidney and scales. The fish liver showed significantly direct relationship with the metal's toxicity of water. The correlation coefficient between the metals toxicity of *Cirrhina mrigala* kidney and liver was positively significant. Therefore, at the Baloki Headworks, there has been observed the alarming situation of toxic metals which are necessary to be monitored regularly.

SCREENING OF PATHOGENIC *E. COLI* FROM VARIOUS SAMPLES OF DAHI BHALLA, FRUIT CHAT AND FISH COLLECTED FROM DIFFERENT AREAS OF LAHORE

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Present work was based on bacterial contamination of food and meat(fish) that is very common due to unhygienic conditions, rough handling, bruising and improper

freezing techniques. Undoubtedly in past 10-20 years the impact of food borne diseases has been over whelming in Pakistan. Microbial screening of all samples of fish, dahi bhalla and fruit chat was done by culturing these on tryptic soya broth, MaConkey agar with sorbitol and novobiocin containing media to detect the presence of pathogenic *E. coli*. The purpose of this study is to develop awareness among people about these pathogenic *E.coli* that causes various digestive and urinary track diseases.

SCREENING OF PATHOGENIC *E. COLI* FROM SAMPLES OF MEAT (BEEF, MUTTON, CHICKEN AND QUAIL) COLLECTED FROM LAHORE

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The present work describes the microbial examination of raw meat samples collected from various localities of Lahore. In the study of 40 samples of raw meat, hygienic quality was checked. All samples were microbiologically analyzed in the research laboratory of Lahore College for Women University. Sampling was done from May to July 2009. Both raw and undercooked meat samples were checked for the presence of pathogenic *E. coli*. Out of 40 samples, 27 showed the positive results having pathogenic *E. coli* growth, whereas 17 samples were found negative.

PESTICIDE RESIDUES IN SHRIMP, FISH AND BRINE SHRIMP DURING POND CULTURE AT GHORABARI (DISTRICT THATTA)

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The pesticide residues were analyzed in waters from Ambro creek and their accumulation in shrimp (*Penaeus merguensis*;s and *P. penicillatus*), fish (*Otothyes ruber*) and brine shrimp (*Artemia*) reared in ponds for a period of four months. Overall eight pesticides viz, alpha-HCH, 4,4-DDE, Dieldrin+2,4-DDT, 4,4-DDT, beta endosulfan and Methoxychlor, Heptachlor exo-epoxide, Heptachlor endo-epoxide and Aldrin have been detected in the shrimp, fish and *Artemia* samples. Out of eight, four pesticides, 4,4-DDT, 4,4-DDE, Dieldrin+2,4-DDT, and Methoxychlor were common to all, whereas, Heptachlor exo-epoxide were found in fish and *Artemia* samples and absent in all shrimp samples. Heptachlor endoepoxide was detected only in *Artemia* samples. Methoxychlor

was found in the highest quantity in all the samples whether it was fish, shrimp or *Artemia*. It has been found that not all the pesticides present in water were accumulated by animals. The accumulation rate and type of pesticides both varied with animal. The maximum quantity and number of pesticide residues was found in brine shrimp, *Artemia*; the fish and shrimp accumulated fewer numbers of pesticides with lesser quantity. After four months rearing, the accumulation of pesticides by shrimps and fish was found to be within the permissible limit since none of the detected pesticides seem to exceed their allowable daily intake (ADI).

CLINICAL MANAGEMENT OF RECTAL PROLAPSE ASSOCIATED WITH PYOMETRA AND USE OF ESTROGENIC COMPOUNDS IN NON-DESCRIPT BUFFALO

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In rectal prolapse, one or more layers of the rectum protrude through the anus due to persistent tenesmus associated with intestinal, anorectal, or urogenital disease. Prolapse may be classified as incomplete, in which only the rectal mucosa is everted, or complete, in which all rectal layers are protruded. Causal factors include severe enteritis, endoparasitism, disorders of the rectum (e.g. foreign bodies, lacerations, diverticula, or sacculations), neoplasia of the rectum or distal colon, urolithiasis, urethral obstruction, cystitis, dystocia, colitis, and prostatic disease. Perineal hernia, or other interruption of normal innervation of the external anal sphincter, may also produce prolapse. Animals of any age, breed, or sex may be affected. The use of estrogens as growth promotants, or accidental exposure to estrogenic fungal toxins, may also predispose large animals to rectal prolapse. The present report describes a buffalo with rectal prolapse associated with pyometra and use of estrogenic compounds. The clinical signs, diagnosis based on clinical findings and underlying causes are discussed. The five years old pleuriparous, non descriptive buffalo was presented to Veterinary teaching Hospital, Department of Clinical Medicine and Surgery, College of Veterinary and Animal Sciences Jhang with eversion of rectal mass. According to the history from the owner, animal was normally parturated to give a birth of female buffalo calf seven days before. Placenta was retained and local quack tried to remove the retained placenta, but failed to do it properly which resulted into pyometra and rectal prolapse as a result of consequent straining. Animal was referred to Veterinary Teaching Hospital, Department of Clinical Medicine and Surgery, College of Veterinary and Animal Sciences Jhang for clinical management of prolapse mass of rectum. On recto-clinical examination the buffalo had severe tenesmus; everted rectal mass which was soiled with dirt, faeces and straws. The prolapse mass was

inflamed, edematous, lacerated with necrosis of rectal mucous membrane. On physical examination animal was found to be depressed, anorexic and restlessness. The buffalo was treated as an emergency case under epidural anesthesia (lignocaine hydrochloride 2% @ 5 ml). The necrosed mucous menibrane, blood clots, faeces and straws were completely removed from the everted mass. The prolapsed portion was washed with mild antiseptic solution (potassium permanganate) and then painted with 50 mlliquid paraffin as lubricant for easy reposition. The prolapse mass was repositioned initially by applying gentle palm pressure and complete reposition was achieved by applying arm pressure through anus. To avoiding recurrence of prolapse, rope truss was applied. After reposition of the prolapsed mass, parental and oral therapy was given as Injection Emofloxacin 100 mg/ml (encure 10 %, @7.5mg/kg bwt, VM), injection. Meloxicame (Diclostar, star laboratories Lahore,@1.8 mg/kg bwt, 11M), injection vitamin B.complex (orient pharma, Lahore@ 20 ml) D.C.P powder was given 100 grams daily. Case showed excellent response to the treatment, as prolapse did not recurs after completion of treatment. Rope truss was removed after completion of five days of treatment. Amass et al (1995) reported that use of estrogens predispose to rectal prolapse, so this case supports the findings as animal was treated with some estrogenic drugs to evacuate the pyometric pus. The condition can be corrected with favorable prognosis if treatment is initiated at early stage to avoid much injury to organ (Noakes *et al.*, 2000).

COMMON DISEASED CONDITIONS AND MANAGEMENTAL PRACTICES OF DONKEYS IN D.G. KHAN

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An estimated 4.27 million donkeys live in Pakistan and 2.23 million of this number is found in Punjab (Livestock census 2006). They play a vital role in rural economics through the provision of draught power and transport. Donkeys are contributing the major proportion of readily available transport needs of poor women and men living in hostile environments, enabling them to integrate into social and economic processes (Fernando and Starkey 1996). To thrive on poor quality minimally supplemented feeds has also made them popular in environments where feed shortages can seasonally become a critical problem. Donkeys have been reported to survive better under drought condition than any livestock species due to their small body size and low dry matter intake requirements minimizing their water and maintenance needs in arid and semi arid areas (NRC 1984). In Pakistan as in other developing countries, one of the most important problems in promoting donkeys is the lack of knowledge about their socio-economic status, husbandry and health needs. Donkeys are mostly used for transport.

They pull carts and carry goods, loads such as, bricks, gravel, sand, fuel in the form of wood for burning in the houses and drums of diesel oil for running the soil water pumps. Similarly they are the source of transportation for carrying agriculture products from main market to remote villages and vice versa. In the northern part of D.G. Khan District, there are about 40 brick kilns and about 1200-1500 working equines. Here the donkeys are used to transport bricks, as they are firm-footed and suitable for carrying freshly prepared bricks to the kilns. These donkeys carry about 45-55 bricks of freshly prepared clay. Each brick weighs about 2.6 kg. The animals work for about 12 hours a day, six days a week. Donkey owners have a small stable for their donkeys. The floors of these stables are always wet and muddy. The mangers are made of clay, and there is no proper ventilation in the stables. Very few owners provide water troughs for their animals in the stables and where troughs are present, they are usually very dirty. The dirtiest utensil in the house is used as drinker for donkeys. Donkeys are not shod and their hooves are not properly cleaned. The hooves are commonly full of mud, dung and small pieces of gravel. The animals are under-fed and debilitated. They are provided with wheat straw or rice husks which contain few nutrients. They are neither fed with green fodder nor given any salt, despite the fact that they work for 12 hours a day. They are never groomed. The donkeys are heavily infested with endoparasites. Animal owners are very poor. They cannot afford to deworm their animals regularly. They are also uneducated so they have no awareness regarding the health, housing, and feeding of their animals. The most frequently encountered problems were colic, coughing, back and hind leg sores. Rolling and other signs associated with colic or tympany, which may also be related to high parasite burdens and impaction. Wounds especially on the back and hind legs, were third cited donkey health problem. Other diseases that were mentioned included diarrhoea, emaciation, inappetence, teeth and lacrymation. Government Veterinary hospital is present in almost each union council, which is headed by a veterinarian, with one veterinary assistant. The hospital provides free treatment to debilitated, injured and diseased animals. The veterinary Hospital understands that the owners of the animals are very poor. Bearing these points in mind, field tour program are arranged to deworm and treat these animal in remote rural area. A mobile veterinary Hospital headed by veterinarian visits the field clinic every day and treats the patients. Despite the great contribution made by donkeys to the daily life of rural people, they suffer the dual negative impact of low social status and poor management. Improvement of management practices, particularly treatment and control of hind legs/ back sores by use of proper harnesses as well as feed supplementation are required to enable better performance of donkeys. Virtually all the owners are also extremely poor, which makes any improvement in the state of their donkeys very unlikely. Ignorance associated with poverty is very difficult to overcome. An expanded program should be started for education of donkey users, establishment of field clinics for indoor patients, husbandry training for the owners of the admitted animals and provision of more mobile teams to cope with disease conditions.

SERUM ADIPONECTIN RESPONSES IN PREGNANT WOMEN DURING DIFFERENT PHASES OF PREGNANCY AND POST DELIVERY

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Adiponectin is an adipose tissue-derived 244 amino acid protein that is a key modulator of insulin action and glucose metabolism. Adiponectin levels are inversely correlated with insulin resistance. In early pregnancy, insulin secretion increases, while insulin sensitivity is unchanged, decreased, or may even increase. However, insulin resistance develops in late gestation while maternal adiponectin levels decline. Normal pregnancy physiology comprises changes that may affect adiponectin levels suggesting that the role of adiponectin in pregnancy is a source of interest. The present study was conducted to measure serum levels of adiponectin in non-pregnant, pregnant <20, 20-30 and >30 weeks of gestation) and post delivered women in order to establish the standard serum levels of adiponectin during normal pregnancy and post delivery. A total of 75 blood samples were collected (15 in each group) with minimum 8hrs overnight fast. The serum adiponectin hormone level was estimated by Enzyme Immunoassay (EIA) in all of the blood samples. In the present study, adiponectin has shown average values of 12.04 ± 0.75 $\mu\text{g/mL}$, 14.57 ± 0.44 $\mu\text{g/mL}$, 12.51 ± 0.87 $\mu\text{g/mL}$, 10.66 ± 0.59 $\mu\text{g/mL}$ and 10.33 ± 0.35 $\mu\text{g/mL}$ in non-pregnant, pregnant <20, 20-30, >30 weeks of gestation) and post delivered women, respectively. In overall comparison, the serum adiponectin levels are comparable among non-pregnant, pregnant (<20, 20-30, >30 weeks of gestation) and post delivered women. Serum adiponectin levels change significantly during pregnancy with an initial increase in the first part of pregnancy to maximum levels at mid-gestation, followed by a decrease throughout the second half of pregnancy and this decrease has been found to persist even in the post delivered women. Adiponectin is upregulated during adipogenesis and downregulated in insulin-resistant state. The pregnancy-mediated changes in adiponectin were strongly correlated with basal insulin levels and insulin sensitivity. The relationship between adiponectin and insulin sensitivity was related to the decreased insulin regulation of glucose utilization.

QUANTITATIVE PROFILING OF SERUM PROTEIN FRACTIONS IN YOUNG OBESE HUMAN

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Study concerns the investigation of serum electrophoretic protein fractions in obese persons (BMI > 30 kg/m²) in comparison to control individuals (BMI ranging

between 18.76-21.45 kg/m²). Serum samples of 27 adult males and females of ages ranging 20-40 years were selected. Height and weight of subjects were taken carefully before sampling in order to calculate their BMI. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed to study serum protein fractions. The data was analyzed statistically using student 't' test to find out enhancement or reduction and appearance or disappearance of particular protein fractions in obese subjects when compared to control group. Fourteen protein fractions were detected that were ranging between 152-15 kDa. Non significant elevations were observed in 114 and 30 kDa protein fractions, while significant elevations were observed in 38, 26 and 20 kDa protein fractions. Non significant reductions were observed in 34 and 17 kDa fractions, while significant reductions were observed in 152,131,76,66,49 and 43 kDa protein fractions. Protein fraction of 15 kDa observed in normal subjects, did not appear in any of the obese individuals. Pattern of serum protein fractions, suggest that some fraction alterations in obese individuals are strong indicators of their roles in alteration of body mass. The absence of 15kDa protein in obese individuals suggests it to be the marker protein for obesity. Although, further investigation on large scale population is needed for much enhanced and better understanding of alterations in protein profile of obese individuals.

PROGNOSTIC SIGNIFICANCE OF SERUM PROTEIN PROFILE IN EARLY DIAGNOSIS OF THYROID DYSFUNCTION IN HUMAN POPULATION

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Abnormalities in the serum proteins have been described in several disorders of thyroid function. The present study was, therefore, planned to investigate the relationship of thyroid disorders with protein profile and the prognostic significance of Marker/Novel protein(s) in early diagnosis of thyroid complications in our local population. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was employed for studying serum protein profile of euthyroid healthy controls (group 1, n=18) and newly diagnosed severe hypothyroid patients (group 2, n=18). The study was further extended to the hypothyroid patients which were exhibiting euthyroid profile on medication, *i.e.*, thyroxine (group 3, n=9). Gels were photographed and quantified for various protein fractions by Gene Genius Bio-imaging Gel Documentation System. The data was analyzed statistically using Student t-test and employed in finding the variations in particular protein fractions for comparison among the patients and healthy subjects. Fourteen protein fractions ranging between 141-10kDa were detected in group 1 and group 2, whereas, fifteen protein fractions ranging between 141-10kDa were detected in group 3. Protein fraction of 109kDa was newly expressed in group 2 although the fraction did not manifest in group 1. On the other hand, protein fraction of 35kDa expressed in

group 1 was entirely absent in group 2 subjects. Moreover, in group 2, protein fractions of 77, 39, 35, 32, 23 and 19kDa indicated significant ($p=0.0000$) reductions of 24, 26, 100, 17, 14 and 26%, respectively, as compared to euthyroid controls, whereas, protein fractions of 125, 109,66, 14, 11 and 10kDa showed significant elevations of 48, 100,44, 38, 21 and 60% in the same group as compared to group 1. In group 3 (euthyroid on medication but with primary diagnosis of hypothyroidism) protein fractions of 141, 48, 39,35,32,23, 1'9, 14,11 and 10kDa exhibited significant elevations of 7, 38, 27, 100, 10, 13,41,23,8 and 28%, respectively, as compared to group 2.

CLONING OF CUS - OPERON REGULATORY REGION (*cusSR*) FOR COPPER HOMEOSTASIS OF *KLEBSIELLA PNEUMONIAE*

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Copper is an essential trace metal for both prokaryotes and eukaryotes, and associated with various metal enzymes which play many essential roles in the living system. But excess of the metal is very toxic and even lethal for living organisms. In microorganisms the intracellular level of copper is maintained by many genes working as an operon and induced by the presence of copper in their environment. One such operon is called *cus* operon regulated by *cusRS* two component system in *Klebsiella pneumoniae*. This *cusS* (sensory region) and *cusR* (regulatory region) autoregulate the expression of structural as well as regulatory part of the *cus* operon by binding of CusR protein to the bidirectional promoter part of the operon. We have isolated the genomic DNA from some previously characterized strains of *Klebsiella pneumoniae* and amplified the whole regulatory *cusRS* region of almost 2.2kb through PCR. We also have inserted this PCR product into cloning vector pTZ57R/T after gene clean and transformed this recombinant vector into a host organism DH5a. We will determine the promoter strength by cloning of some suitable reporter genes in front of this promoter and estimating their expression.

SERUM RESISTIN LEVELS IN PREGNANT AND POST DELIVERED WOMEN

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Resistin a (114-aminoacid polypeptide) has a relative mass of 12 kDa, and belongs to a family of cysteine-rich C-terminal domain proteins called resistin-like molecules (RELMs). Human pregnancy is characterized by a series of metabolic changes that promote adipose tissue accretion in early gestation, followed by insulin resistance and facilitated lipolysis in late pregnancy. Pregnancy is associated with higher plasma

concentrations of resistin than in the non-pregnant state and a further increase occurs during the third trimester. The present study was planned to measure serum levels of resistin in healthy non-pregnant, pregnant <20, 20-30 and >30 weeks of gestation) and post delivered women. Both control and experimental samples were analyzed for resistin hormone. Hormonal study was carried out by Enzyme Immunoassay (EIA) for resistin hormone in all of the blood samples. The resistin has shown average values of 6.11 ± 0.15 , 8.29 ± 0.23 , 9.98 ± 0.16 , 12.86 ± 0.17 and 11.48 ± 0.22 ng/ml in non-pregnant, pregnant <20, 20-30, >30 weeks of gestation) and post delivered women, respectively. The principle findings of the study reveal that the serum resistin levels are comparable among the study groups. The level has significantly increased in pregnant <20, 20-30, >30 weeks of gestation) and post delivered women as compared to non-pregnant women but decreased significantly in post delivered women as compared to pregnant women (>30 weeks of gestation). Alterations in the maternal plasma concentration of resistin during pregnancy could contribute to metabolic changes of pregnancy. The increase in serum resistin in late pregnancy could be a systemic signal to reduce insulin, sensitivity often developed in later stages of pregnancy.

**EFFECT OF *CERIOPS TAGAL* (Perr.) C.B.ROB. STEM BARK EXTRACT
AGAINST SOME ATMOSPHERIC FUNGAL ALLERGENS**

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Fungi are the causal agents of systemic mycoses, superficial mycoses, other opportunistic infections and allergic disorders. These problems are controlled by synthetic antifungal drugs which adversely affect the liver, kidney and gastrointestinal tract of human beings and increase the hepatic enzymes up to dangerous level also. Keeping in the view, the mangrove plant *Ceriops tagal* (Rhizophoraceae) was selected which is found along different coastal areas of Sind and Balochistan. In vitro, the ethanolic extract of *Ceriops tagal* stem bark was tested against seven allergenic fungi viz., *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *C. herbarum*, *P. notatum*, and *Saccharomyces cerevisiae*, using five solvents DMSO, DW, chloroform, ethanol and acetone at three 2000, 4000 and 6000 ppm doses. Poisoned food technique (modified) was employed during the study. For positive control, two synthetic antifungal drugs, miconazole against *A. alternata*, *Cherbarum*, *P. notatum* and *S. cerevisiae* and amphotricin-B against *Aspergillus spp.* were used. Percent inhibition in the growth of fungi was found in the descending order of *A. Alternata* ($60.33\% \pm 6.8$), *Aniger* ($55.91\% \pm 4.00$), *Aflavus* ($52.00\% \pm 4.79$), *Afumigatus* ($39.00\% \pm 6.97$), *Cherbarum* ($33.75\% \pm 5.99$), *P. notatum* ($31.82\% \pm 13.33$) and *S. cerevisiae* ($17.00\% \pm 13.09$). Overall, dose dependent tendency in the increase or decrease in the growth of fungi was noted during

the study. The data was compared with negative control and most of the results were found as significant ($p < .05$).

QUORUM SENSING, BIOFILM FORMATION AND INHIBITION IN DENTAL UNIT WATER LINES

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Dental unit water line (DUWL) contamination by opportunistic pathogens has its significance in nosocomial infection of patients, health care workers and life-threatening infections to immunocompromized persons. Recently, the quorum sensing (QS) system of DUWL isolates has been found to affect their biofilm-forming ability, making it an attractive target for antimicrobial therapy. In the present study, the effect of two quorum-sensing inhibitory compounds (patulin; PAT, penicillic acid; PA) and EDTA on planktonic growth, AI-2 signaling and *in-vitro* biofilm formation of *Pseudomonas aeruginosa*, *Achromobacter xylosoxidans* and *Achromobacter* sp. was monitored. *Vibrio harveyi* BB 170 bioassay and crystal violet staining methods were used to detect the AI-2 monitoring and biofilm formation in DUWL isolates respectively. The *V. harveyi* BB 170 bioassay failed to induce bioluminescence in *A. xylosoxidans* and *Achromobacter* sp. while *P. aeruginosa* showed AI-2 like activity suggesting the need of some pretreatments prior to bioassay. All strains were found to form biofilms within 72 h of incubation. The QSIs/ EDTA combination have isolate-specific effects on biofilm formation and in some cases it stimulated biofilm formation as often as it was inhibited. However, detailed information about the anti-biofilm effect of these compounds is still lacking.

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF β -1,4-XYLOSIDASE FROM A NOVEL *HUMICOLA* STRAIN

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An indigenous *Humicola* strain 692 produced maximum productivity (17 IU/L.h) of extracellular β -xylosidase on 0.2% cellobiose after 72 h fermentation at 45° C. 3%

wheat straw was better inducer than carboxymethyl cellulose (CMC, an inducer of β -xylosidase). 1, 4- β -xylosidase was purified from *Humicola* strain to homogeneity level by using a combination of $(\text{NH}_4)_2\text{SO}_4$ precipitation, gel filtration and ion-exchange chromatography. The purified enzyme possessed a molecular weight of 56 kDa, optimal pH of 6.5 and an optimal temperature of 60° C. Temperature- and pH-dependent kinetic parameters for β -xylosidase hydrolysis were determined. Enzyme exhibited stability up to 80° C and over a pH range of 6.0-9.0. Purified enzyme showed a half-life of 90 min at 60° C. It exhibited Michaelis-Menten kinetics with K_{cat} of 1475 min^{-1} and K_m of 4.8 mg/mL at 60 °C. Magnitudes of activation energy enthalpy, entropy and Gibbs free energy demand for active catalysis and inactivation revealed that it was quite thermo-stable. These specific characteristics make it a candidate for its futuristic large-scale production in our country.

GUANIDINOACETATE N-METHYLTRANSFERASE DEFICIENT MOUSE AS A MODEL FOR NEUROPROTECTIVE ROLE OF CREATINE FOLLOWING NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY

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The creatine/phosphocreatine (Cr/PCr) system is essential for cellular phosphate coupled energy storage and production, particularly in tissues subject to high metabolic demands such as the heart, skeletal muscle and central nervous system. In these tissues the Cr/PCr system is the first system recruited when a cell is energetically challenged, reflecting a special significance in cellular viability. Creatine is synthesized in a two step reaction involving Arginine:Glycine Amidinotransferase (AGAT) and Guanidinoacetate N-Methyltransferase (GAMT) respectively. Creatine may be taken up by cells via the creatine transporter (CrT). Defects in any of these enzymes or transporters will lead to systemic creatine deficiency and severe neurological disease. Transgenic mouse models of cerebral creatine deficiencies are ideal disease models to study the neuroprotective effects of creatine following neonatal asphyxia, which is associated with significant morbidity and mortality. The aim of this study is to investigate the effects of creatine supplementation in GAMT mouse models following hypoxic and ischemic (HI) events. Knockout female GAMT and their wild type littermates were used in this study which has short term and long term phases. At postnatal day 7, pups underwent right carotid artery ligation followed by hypoxia (8% O₂) for 20 min (short term phase). A series of behavioural test batteries, biochemical analysis of plasma/urine and brain infarct measurements were carried out after H/I events. Following weaning, mice were separated in various groups on the basis of their genotype and diet supplementation (creatine free diet or 2% creatine supplemented diet) for 10 weeks. Various neurological

tets like morris water maze, rota rod, open field, biochemical analysis of blood plasma and urine and whole brain histology were performed to observe the effect of diet following HI events. Separate group of mouse with out hypoxic ischemic insult, for all treatments, were used as control for short and long term experiments. During short term experiments, GAMT wild type pups performed significantly better than knockouts to perform various behavioral reflexes. The performance of knockouts was even worst following hypoxic ischemic insult. During long term phase, we observed that creatine supplementation improves the performance of both wild type and knockout mice in all HI as well as in no HI treatments. Knockout mouse even performed better than wild type littermates in some experiments. Performance of wild type as well as knockout GAMT mouse was significantly affected in experimental groups fed on creatine free diet. Creatine supplementation improves the over all performance of GAMT knockout as well as wild type mouse. GAMT knockout mouse is an ideal model to observe the effects of creatine supplementation as these mouse completely lack the creatine in their body. Creatine has a neuroprotective effect following the neonatal hypoxic ischemic insult and it can be used to reduce the damages of asphyxia.

DETECTION OF CYTOMEGALOVIRUS BY PCR AND SIMPLE ELISA IN PREGNANT WOMEN

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To find out the frequency of HCMV active infection in asymptomatic pregnant women by simple ELISA (TORCH) and PCR, samples were collected from the gynecology wards of Lady Reading Hospital Peshawar and Khyber Teaching Hospital Peshawar NWFP. A total of 100 pregnant women were selected, detailed clinical history was recorded from each women and informed consent was obtained. ELISA was performed for each sample. DNA was extracted from whole blood by using DNA extraction kit and DNA was amplified by nested PCR. Specific HCMV bands were analysed after gel electrophoresis. The overall prevalence of HCMV infection in 16-40 year-olds was 10% and number of abortion noted was 0-5 times. In women with greater number of abortion active infection of HCMV was observed. While most of the women with 3 time abortions were found positive for antibodies against CMV, HSV, Rubella virus and Toxoplasma. Force of infection was significantly higher among age group 20-40 years. It is concluded that HCMV is one of the causative agent of abortion in pregnant women and that hundreds of HCMV infections are present in pregnant women and numerous unborn babies are at risk for serious disabilities. Moreover, PCR is more sensitive, reliable and accurate method for detection of HCMV infection during pregnancy.

**A STUDY ON SERUM BIOCHEMISTRY AND HEMATOLOGICAL
PROFILING OF BLUE ROCK PIGEON (*COLUMBA LIVIA*) IN MULTAN
(PUNJAB: PAKISTAN)**

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Thirty wild blue rock pigeons (*Columba livia*) were caught from Multan city (Pakistan). Hematological values were established for total red blood cells (TRBC), total white blood cells (TWBC), packed cell volume (PCV), hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leukocyte count (DLC). Serum biochemistry values were determined for glucose, urea, cholesterol, creatinine, lactate dehydrogenase (LDH), total protein, alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) as indicator of bird's health and can be used in the future as a reference values in hematology and serum biochemistry of blue rock pigeon .

**RELATIONSHIP OF INSULIN AND TRIIODOTHYRONINE IN IMPAIRED
GLUCOSE TOLERANT AND TYPE 2 DIABETIC PAKISTANI SUBJECTS
WITH NORMAL THYROID FUNCTION**

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A total of 508 euthyroid subjects, diagnosed as impaired glucose tolerant, type 2 diabetic and normal glucose tolerant (150) were sampled and analyzed for metabolites and data was worked out to investigate the relationship of triiodothyronine (T_3) and insulin in the glycemic anomalies. Oral glucose tolerant test (OGTT) was employed to confirm the glycemic status. The subjects were categorized using Diabetes Expert Committee criteria (2003). Thyrotropin (TSH), total triiodothyronine (TT_3), total thyroxine (TT_4) and insulin were assessed by enzyme linked immunoassays (ELISA). Fasting plasma glucose and HbA1c were measured by glucose oxidase and low pressure cat ion exchange chromatography. Homeostasis model of assessment (HOMA-IR) was employed to assess the level of insulin resistance. Anthropometric measurement and habits were recorded. Younger glucose intolerant subjects were highly hyperinsulinemic and insulin resistant as compared to the diabetic group ($p < 0.05$). Serum concentration of TT_3 was significantly low in IGT and diabetic group as compared to NGT ($p < 0.05$). Serum concentration of TSH was significantly higher in IGT and diabetic group. A

significant inverse correlation of fasting serum insulin with T_3 prevailed in these subjects ($r=0.425$ $p<0.05$). In multiple regression analysis when insulin was a dependent variable TT_3 and TSH were significant predictors in IGT and diabetic groups respectively $p<0.05$. The homeostatic relationship of insulin and T_3 has been lost with the parallel development of insulin resistance and hyperglycemia in glycemic anomalies. The ratio of insulin and T_3 is important in glucose homeostasis.

STRUCTURAL STUDY OF ERYTHROPOIETIN USING BIOINFORMATICS TOOLS

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Erythropoietin (EPO) is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow. It is produced by the kidney & liver that promotes the formation of red blood cells in the bone marrow. Human EPO consists of 193 amino acids. The gene of EPO is located on chromosome 7 and consists of 576 nucleotides. We analyzed structural and functional domains of erythropoietin by using different bioinformatics tools. The tools from three databases were selected. For primary structure prediction ProtParam, Compute pI/Mw, ScanSite pI/Mw, Radar, REP, REPRO, SAPS, 2ZIP, ProtScale, Protein Colourer and Colorseq were used. For secondary structure prediction HNN and nnPredict were used and for tertiary structure prediction 3Djigsaw and Ramachandran Plot were used. 3D structure was visualized in RasMol, Cn3D and PDB Gmol, webmol and king viewer). Structural domains was identified using DOMAIN 3D. The present study is very useful for recombinant erythropoietin production as well as for new drug development. This work also reveals that there is a lot of difference in results of different online tools.

DEVELOPMENT OF CHLOROPLAST GENOME DATABASE

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Plant chloroplast is the ideal hosts for expression of translate. Chloroplast contains its own genome that transcribes, translate and replicate autonomously. Chloroplast genetic engineering is getting importance day by day because engineering trans gene in chloroplast genome provides unique benefits over nuclear transformation. The sequenced data of Chloroplast genome is increasing day by day. Thirty-five sequenced crops chloroplast genomes data is available on different databases. There is no separate database available for chloroplast genome till now. We initiated a database of chloroplast

genome. Chloroplast database was designed by using MySQL database server. This database is a relational database with a user-friendly interface and provides tools to aid the analysis of chloroplast genome sequences. Tools and techniques are used in this project are, Microsoft Visual Studio 2005, Microsoft SQL Server 2000 IIS (Internet Information Services), Adobe Photoshop, HTML (Hypertext Markup Language), CSS (Cascaded Style Sheets). This online database is a small effort, in the genome world. It will provide single platform and easy access to the scientists and researchers working in the field of chloroplast biotechnology.

**BILIRUBIN HAS NO SIGNIFICANT EFFECT IN THE CONTROL OF CANCER
IN BOTH MALES AND FEMALES**

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ILYAS

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Bilirubin is the yellow breakdown product of normal heme catabolism. It plays an important role in the protecting our body and in neuronal signaling. We collected and analyzed the data from different hospitals to evaluate the role of bilirubin in reducing cancer. We carried out the comparative study of conjugated and unconjugated bilirubin in both normal and abnormal males and females of different age groups in different hospitals. Statistical analysis has showed that bilirubin do not have any significant effect in reducing cancer.

OrGene: AN ORGAN BASED CATEGORIZED HUMAN GENOME DATABASE

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OrGene is an organ based categorized human genome database developed for the categorization of human genome on the basis of organs. The function of the proteins encoded by these genes also made available. OrGene will expand in future to other organs, genes and cover more useful information of other species. Primers provided for each gene, with their features and conditions given to facilitate the researchers, are useful in PCR amplification, especially, in cloning experiments. Flexible database design, expandability and easy access of information to all of the users are the main features of the database. The database is publicly available at www.orgene.pkstn.com.

STUDIES ON GLAUCOMA IN RELATION TO VARIOUS FACTORS AND THEIR ASSOCIATES AMONG PATIENTS VISITING EYE OPD NISHTER HOSPITAL MULTAN PAKISTAN

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The present study was carried out to assess glaucoma in relation to different factors and their associates among patients visiting eye OPD Nishter Hospital Multan Pakistan for the period of 12 months from January 2005 – December 2005. Patients of both genders (n=401) were divided into three age groups *i.e.* (age 15-35 years), (age 36-55 years) and (age >55 years) and visual acuity, intraocular pressure, cup-disc ratio, hypertension and incidence of diabetes mellitus were analyzed. Patients were also divided into various groups on the basis of their nature of job and area of origin. The results indicated that 44.88% patients had mild, 25.18% had moderate and 3.99% patients had severe vision loss. 10.22% had no vision while 15.71% patients had normal eye vision. Mature males of age group (36-55) years were suffering maximum with the severe disease (6.36%) compared to old males (5.63%). Among the observed female patients a different trend was observed and old females of age group (56 & above) were suffering maximum (5.31 %) with the disease. Visual acuity disorder was maximum in left eye of both male (22.53%) and female (14.89%) patients of age group greater than 55. Also when severity of disease was compared between both eyes, it was found that left eyes were suffering more severely (12.46 %) as compared to the right eyes (3.99 %). There was highly significant effect of age on intra ocular pressure ($P = 0.000$), cup disc ratio ($P = 0.001$) of left eye and visual acuity ($P = 0.001$) of both eyes among all age groups. Results also demonstrated that 11.5% glaucoma patients of age group >55 years were suffering from hypertension while 15.7% were diabetic with age between 36-55 years.

IN SILICO COMPARATIVE ANALYSIS OF p53 GENE VARIANTS

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Homo sapiens seven transcript variants of p53 gene were taken from NCB!. Translated variant of p53 were used for protein comparative study. Five different bioinformatics tools were used for the comparative analysis of p53 proteins RROTP ARAM tool was especially used to study the physio-chemical properties *e.g.* (composition of amino acid, molecular weight, molecular formula, half life if of each

protein and stability index. Splicing mutation in seven p53 proteins were studied that cause the different types of cancers. Because mainly physio-chemical composition of proteins disturbed that can produce any type of cancer. A standard curve has been plotted between (mw/pI). It helps to detect the p53 protein problem in their function. CLUSTALW2 tool used to study the evolutionary relationship between the p53 proteins. SOPMA tool used to compare the secondary structure of p53 proteins that was especially used to study α -helix, B turns, random coils, extended strands. Bands were used to compare the p53 proteins. Main purpose of this tool was to study the folding points in the p53 proteins and conducted the effected protein portions. A standard curve was plotted indicate the proportion of coils, B turns, α -helix. DisEMBL used to identified the un structural and disordered segments of p53 proteins. In future this study will be helpful to predict disordered regions within p53 proteins. p53 detection software will be developed for local and low cost diagnose of cancer in Pakistan

COMPARATIVE STUDY OF VARIOUS DRUG EFFECTS AGAINST TUBERCULOSIS

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Tuberculosis (TB) is one of the most common and deadly infectious diseases caused by *Mycobacterium tuberculosis*. TB infection usually occurs initially in the upper part (lobe) of the lungs. Active TB was treated with a combination of medicines along with isoniazid, Rifampin (Rifadin), ethambutol (Myambutol), and pyrazinamide, these are the drugs commonly used to treat active TB in conjunction with isoniazid (INH). Many drugs are helpful for prevention and cure of tuberculosis but the purpose of present study was to check the efficacy of these drugs against TB patients, data was collected from TB Hospital Faisalabad, Allied Hospital and Civil Hospital and investigated by applying Mantox test using Myrin-P tablets (Ethambutol HCL, Rifampicin, Isoniazid, Pyrazinamide). Patients of TB before and after the treatment were compared during the time period of Mantox test. Correlation was found between age groups. and gender. The Ethambutol, Isoniazid and Rifampicin were significantly effective because p-value were 0.002, 0.000 and 0.000 respectively. While Pyrazinamide was not significantly effective because p-value was 0.07. This analysis also showed that antisera. is not statistically significant as p-value is 0.416 which means that antisera categories were not dependant on gender categories (*i.e.* the attributes are independent). AU drugs were significantly effective but Isoniazid is most efficient drug for the TB patients and Pyrazinamide is least efficient. In future the genes that are involved in causing TB should be well investigated. Scientists should study its particular pathogenic motif. So, that a target based drug can be made that could cure TB.

MOLECULAR PHYLOGENY AND RECOMBINATION OF BETASATELLITES ASSOCIATED WITH BEGOMOVIRUSES

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In Asia the majority of begomoviruses are monopartite and associate with a group of symptom modulating satellites ssDNA molecules known as betasatellites. Begomoviruses are exclusively transmitted by the whitefly *Bemisia tabaci*. These viruses can cause significant diseases to numerous crops including, for example, cotton. Betasatellites molecules have a singular gene which is functional in complementary sense (WC I) that encodes all functions identified so far. Moreover the satellite molecule has a region of sequence rich in adenine residues (A-rich), the function of which remains unknown, and an approx. 200bp sequence conserved between all betsatellites (known as the satellite conserved region). The SCR is believed to be important in trans-replication by the helper begomovirus. Although the phylogeography and recombination of begomoviruses has been examined in some detail, little is known about recombination of betasatellites and what this can tell us about the evolution and spread of these important sub-viral molecules. We have examined 213 publicly available betasatellite sequences originating from Asia and China for evidence of inter as well as intraspecific recombination. These show extensive evidence for recombination between geographically widely separated species. In particular, the evidence for recombination involving the A-rich sequence and the SCR is strong. The significance of these findings to our understanding of the evolution of these molecules and their geographic spread will be discussed.

IN SILICO ANALYSIS OF AQUAPORINS

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Aquaporins are proteins embedded in the cell membrane that regulate the flow of water. They are "the plumbing system for cells". Aquaporins are integral membrane proteins from a larger family of major intrinsic proteins (MIP) that form pores in the membrane of biological cells. Conserved Motifs were identified within the Aquaporin family for function recognition. Conservation Analysis of Aquaporin proteins was performed by aligning protein sequences using CLCbio Genomics Work bench. SBASE

was used for the Identification of protein domains and function. Aminocomp was used to find Composition of Amino Acids and their pl. The amino acid composition of Aquaporin protein family was checked to see similarity between these proteins. An important issue when trying to understand protein function is to know the actual structure of the protein. PSIPRED software was used for this purpose. A tool in Visual Basic was developed for the search of specific motifs of Aquaporin protein family especially in Arabidopsis Thaliana. Domain prediction of Aquaporin protein family showed that Major intrinsic protein - like domain is present throughout the family members. Secondary Structure Analysis and Composition of Amino Acids showed little divergence in these proteins. The over all analysis of all the members of this protein family showed that they belong to same family based upon similarity in structure, but are involved in transport of different molecules, due to low similarity at sequence level. The importance of the said findings and their future perspectives to best of our understanding will be discussed.

IN SILICO COMPARATIVE ANALYSIS OF MAMMALIAN *Tp53* GENE USING BIOINFORMATICS TOOLS

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The 20 kb of *p53* gene is located on chromosome 17 (17p13) with base pair ranged from 7,512,463 to 7,531,641. There are 11 exons and very large intron is present between exons 1 and 2. Exon 1 is non-coding in the human *p53* and this region could form a stable stem-loop structure which binds tightly to wild type *p53* but not to mutant *p53*. Comparative analysis of *TP53* gene from 17 different species of *mammals* was performed by using following tools Align (EBI-pairwise alignment tool), ClustalW2 (a multiple alignment tool), Primer3 (primer recognition tool in a given sequence), NetPrimer (a tool used to determine the percent efficiency of primers found by Primer3) and NEB cutter (a Restriction analysis tool). The *TP53* (tumor suppresser gene) sequences from these species were obtained from NCB!. These sequences were subjected to multiple alignment. Sequences from most closely related species to *Homo sapiens* i.e., *Macaca mulatta* and *Bos taurus* with the similarity of (80.8%) and (64.4%) respectively, were used to design primers. The primers were then analyzed to determine their efficiency and could be further used in PCR which could be used for diagnostic purposes especially in malignant diseases. Restriction analysis was performed to find restriction sites and restriction enzymes. The restriction analysis is very important in biotechnology as biochemical scissors and biochemical markers and even could be used for gene therapy.

**IN SILICO COMPARATIVE ANALYSIS OF MYCOBACTERIAL SULFOLIPID-
L BIOSYNTHESIS PATHWAY ENZYMES FOR DRUG TARGET
IDENTIFICATION**

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Tuberculosis is one of the most serious health problems, as globally, around one third of the population has been infected with *Mycobacterium tuberculosis*. The human pathogen *Mycobacterium tuberculosis* is thought to control the human immune response with a variety of unique exotic lipids which can be the most perfect targets for drugs against tuberculosis. The main objective of the study was *in silico* identification of drug target in Sulfolipid-l (SL-l) biosynthesis pathway of *Mycobacterium tuberculosis*. Out of five known proteins of this pathway, only four (MmpL8, StfO, PapA1 and PapA2) were selected as putative target, showing non-significant similarity with human proteome using blastp 2.2.21 program with E value greater than 0.01 except one protein, Pks2, that showed significant similarity with human proteome. Among these targets only MmpL8 (a membrane protein) was found to be involved in tuberculosis infection and its 3D structure was predicted in this study to find the structural parts that could be targeted by the drugs. The 12 TMDs of MmpL8 were modeled properly using AcrB and MexB as template by MODELLER 9v7 tool. Model gave a putative image of its structural conformation that helped us in finding the structural parts. In SL-l pathway, MmpL8 could be used as drug target and If the 2nd large non- TMD of MmpL8 is blocked, it is possible to block the function of MmpL8 making the *Mycobacterium tuberculosis* attenuated leading to the cure of tuberculosis infection. Such attenuated mycobacterium pathogens could be used in vaccines production.

**PREVALENCE OF MASTITIS AND BACTERIOLOGY OF MASTITIC MILK IN
DROMEDARY CAMELS IN DISTRICT JHANG**

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Quarter milk samples (n=200) from 50 she-camels were examined to study the occurrence and causes of mastitis in traditionally managed camels in District Jhang. Milk samples collected from 50 she-camels were tested for subclinical mastitis by Surf Field Mastitis Test (SFMT). Overall prevalence of subclinical mastitis was 38% and out of 200 quarter samples only 56 (28%) quarters were having clinical mastitis or reacted positive.

In 12 (63.16%) she-camels, all the four quarters got infected, while, in 3 (15.78%) she-camels only one quarter was affected. The percentage of she-camels having two or three mastitic quarters was 10.53 (2 she-camels). Of the 56 milk samples collected from 19 she-camels suffering from clinical (n=5 quarters) and subclinical mastitis (n=51 quarters), 53 (94.64%) yielded growth of different micro-organisms when cultured on Esculin blood agar and MacConkey's agar plates. A total of 84 isolates of 7 different microbial species were recovered. *Staphylococcus spp.* was the most frequently recovered bacterial species accounting for 38% of all isolates, followed by *Streptococcus spp.* (23%), *Escherichia coli* (14%), *Bacillus species* (10%) and *Corynebacterium* (6%). *Candida species* and Yeast accounted each for 4.5 percent of 84 isolates. Improved hygienic measures and providing extension services to the camel herders can reduce losses due to mastitis.

COMPARATIVE PROFILE OF FREE IONS IN SPIDERS OF CITRUS

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Most common local occurring spiders were selected for the determination of free ions of venom and hemolymph. The comparison between free ions found in venom and body fluid of these species showed characteristically significant differences. High concentration of K⁺ was found in venom as compared to the body fluid. K⁺ and Ca²⁺ concentrations were lower in females as compared to their male counterparts. Only females of the genus *Myrmarachne* had a low concentration of K⁺ and Na⁺ while *Sparassidae* sp. had highest K⁺ contents that were significantly different. There was a non significant difference in Ca²⁺ contents of venom and hemolymph. The differences and variations were mostly species specific. Na⁺ contents w

MASTITIS-A POTENTIAL THREAT TO DAIRY CAMEL

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Camel is a unique animal species on this planet serving the humanity since centuries in Asia and Africa particularly in the arid and semi arid regions. Camel is the animal which has capability to survive and produce in the harshest environment of the world where other dairy animals can't. It produces maximum quantity of milk (even more

than 5000 liters) and for longer periods (8-18 months) than that of other dairy animals. Mastitis is considered one of the most costly diseases of the world mostly found in cattle, buffaloes, sheep and goats. In recent past now this problem has been reported in camel also. Its incidence in sub-clinical form is comparatively higher in the dromedary camel (*Camelus dromedarius*) as compared to Bactrian camel (*Camelus bactrianus*). Like other animals it causes economic losses in terms of low milk production, low milk quality, shortening the life of lactating camel, increasing treatment charges, premature culling etc. Even some of the losses in camel reproduction are attributed to udder diseases. The commonest bacteria causing mastitis in camels include *Staphylococcus aureus* and *Streptococcus agalactiae*. Moreover, tick infestation and application of unhygienic antisuckling devices by the pastoralists have been identified as the predisposing factors. Somatic cell count (SCC), California Mastitis Test (CMT) and level of Adenosine Triphosphate(ATP) were found common techniques for the mastitis detection. Globally the losses owing to mastitis amounts 53 billion dollars in animals excluding camels. The present paper highlights the mastitis, its causes and common detection techniques followed by some practical measures for its control. As a whole mastitis control will not only lead to improve the camel milk productivity through sound and healthy udder but will bring improvement in the pastoralists lives through increased milk supply for their own families as staple diet.

TRADITIONAL, NOVEL FERMENTED AND FUNCTIONAL DAIRY PRODUCTS IN BANGLADESH

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Fermented foods, exclusively and traditionally prepared from milk or its byproducts, are very popular in Bangladesh. Almost all of these foods are functional in nature. Among these functional fermented products dahi, yogurt, acidophilus milk, lassi, borhani, matha, fermented whey drinks and cheese are noteworthy. These products are widely manufactured throughout Bangladesh. Out of the entire fermented products available, people of all classes and age prefer dahi (usually sweet curd) either as a part of the daily diet or as a refreshing dessert. Dahi is prepared by using a mixed culture of *Streptococcus lactis*, *Streptococcus thermophilus*, *Streptococcus cremoris*, *Lactobacillus bulgaricus*, and *Lactobacillus plantarum*. Nutritionally, dahi is nearly complete food since it is made from milk and additional nutritional feature also formed due to use of *Streptococcus* and *Lactobacillus* organisms. The metabolic product of these organisms also plays beneficial role in different health aspects like blood pressure, cholesterol content and antimutagenicity. Matha which is prepared from butter milk and sour in taste; a little amount, 0.5% salt may be added to increase the palatability. Borhani is prepared from sour dahi to which various spices are added to give a spicy flavour. Lassi,

a refreshing beverage made from sweet curd, sugar syrup and rose flavour, is liked by all especially during summer when served with ice. Acidophilus milk and fermented whey drinks are new additions to the industry. Cheese, in the name of the "Dhaka cheese", is sold in different cities of Bangladesh. Soymilk is being used as a milk replacer for various dairy products preparation and also for economic calf feeding. Four per cent of the total milk production is used to prepare dahi and 10% of the milk used for the production of all other fermented products, but the amount of milk used for various dairy products is gradually increasing.

EFFECT OF INSECTICIDES AND ESTROGENS AS HORMONAL DISRUPTORS ON CULTURABLE FISH

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Insecticides, androgens and estrogens, in low concentrations, act as hormonal disruptors, affecting different body functions, by either imitating the hormones or blocking their synthesis and activities. Such effects are visible in the general morphology and histology of different target tissues. The present study was designed to examine such effects of insecticides and estrogens on culturable fish. The fish (*Cyprinion watsoni*) was caught from Rumly stream and divided into seven groups of ten fish each. Nova (0.15:0.03mg, levanogestral:ethinyl estradiol) was given to three group in doses of 2.5, 5.0 and 10.0 ng/l, whereas thunder (50 EC dichlorovos) was given to three other groups in doses of 1.25, 2.50 and 5.00 ppm daily for six weeks. The seventh group of 10 fish was maintained as control. The specimens were sacrificed and observed for sex ratio after six weeks. The gonads, liver and kidneys were removed and processed for histological studies. A progressive increase in the number of female fishes was observed with increasing doses, except for 10.0 ng/l nova, though there was more female fish in that group too. The histological observations showed mild to severe necrosis, hemorrhage, free spermatids, aggregation of spermatids and ruptured boundaries in testis in response to both treatments, whereas nuclear degeneration, clumping of cytoplasm, degenerative follicles, necrosis and ruptured boundaries were seen in ovaries dose dependently. The effects on the kidneys were also dose dependent including degeneration of renal tubules, clumping of cells, necrosis and dilation of the renal tubules, hemorrhage and pkynosis. There was an increased hemorrhage, pkynosis, degeneration, vacuolation, malenomacrophage centre, atrophy and clumping of cells in liver with increasing insecticide and hormonal concentrations.

**ISOLATION AND CHARACTERIZATION OF ARSENIC REDUCING
BACTERIA FROM INDUSTRIAL EFFLUENTS AND THEIR POTENTIAL USE
IN BIOREMEDIATION OF WASTEWATER**

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The present study is aimed at assessing the ability of *Klebsiella oxytoca*, *Citrobacter freundii* and *Bacillus anthracis* to reduce arsenate into arsenite. *C. freundii* and *B. anthracis* could tolerate As (V) up to 290 mg/l. *K. oxytoca* resisted As up to 240 mg/l. *K. oxytoca* and *B. anthracis* showed optimum growth at pH 7 while *C. freundii* showed maximum growth at pH 5. *C. freundii* and *B. anthracis* showed optimum growth at 37°C while the maximum growth of *K. oxytoca* was observed at 30°C. *K. oxytoca* and *B. anthracis* were found sensitive against ampicillin while *C. freundii* showed resistance against it. *C. freundii* and *B. anthracis* were sensitive to erythromycin, kanamycine, nalidixic acid, and tetracycline while *K. oxytoca* was found resistant against these antibiotics. All bacterial strains were found to be sensitive to amoxicillin, chloramphenicol, neomycine, oxytetracycline, streptomycine, and polymixin B but all bacterial strains showed resistance against bacitracin. In arsC reductase crude assay *K. oxytoca*, *C. freundii* and *B. anthracis* showed high ability to reduce As(V) into As(III) 78%, 70%, and 84%, respectively. The bacterial isolates can be exploited for bioremediation of arsenic containing wastes, since they seem to have the potential to reduce the arsenate into arsenite form.

**APPLICATION OF QUORUM SENSING INHIBITORY COMPOUNDS
ALONGWITH BIOCIDES ON BIOFILM FORMATION AND GROWTH OF
DENTAL UNIT WATER LINE BIOFILM ISOLATES**

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Dental unit water line (DUWL) contamination by opportunistic pathogens has its significance in nosocomial infection of patients, health care workers and life-threatening infections to immunocompromized persons. Recently, the quorum sensing (QS) system of DUWL isolates has been found to affect their biofilm-forming ability, making it an

attractive target for antimicrobial therapy. However, detailed information about the anti-biofilm effect of these compounds is still lacking. In the present study, the effect of two quorum-sensing inhibitory compounds (patulin; PAT, penicillic acid; PA) and EDTA on *in-vitro* biofilm formation, planktonic growth and AI-2 signaling of *Pseudomonas aeruginosa*, *Achromobacter xylosoxidans* and *Achromobacter* sp. was monitored. Crystal violet staining method for biofilm formation as well as inhibition, while *Vibrio harveyi* BB170 bioassay was used to detect the AI-2 signaling in DUWL isolates. All strains were found to form biofilms within 72 h of incubation. The QSIs/ EDTA combination have isolate-specific effects on biofilm formation and in some cases it stimulated biofilm formation as often as it was inhibited. The *V. harveyi* BB170 bioassay failed to induce bioluminescence in *A. xylosoxidans* and *Achromobacter* sp. while *P. aeruginosa* showed AI-2 like activity suggesting the need of some pretreatments prior to bioassay.

DETERMINATION AND TREATMENT OF ADRENAL INSUFFICIENCY IN PATIENTS WITH SEPTIC SHOCK

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The deficiency of cortisol is associated with increased morbidity and mortality during critical illness and its increased requirements during stress or illness are routinely met by administration of replacement doses. The present study was designed to determine the incidence of adrenal insufficiency (AI) and the effects of glucocorticoid replacement therapy in patients with impaired adrenal function suffering from septic shock. Twenty-one patients with cortisol concentrations <5-20 µg/dl (normal range), admitted to the Medical and Surgical Intensive Care Unit of the Shifa International Hospital, Islamabad were included in the study. The secretory capacity of the adrenal cortex was determined by measuring the levels of cortisol before and after infusions of low (1 µg) and standard (250 µg) doses of adrenocorticotrophic hormone (ACTH) within 24 hours of the diagnosis of the septic shock. Patients with subnormal adrenal responses to ACTH were treated with stress doses of steroids. Out of twenty-one patients, five patients (23.8%) exhibited AI as determined by ACTH stimulation test. Three of them received steroid supplementation with rapid improvement in hemodynamic parameters. Sixteen patients had adequate adrenal responses (AAR) to the standard ACTH infusion. Mortality in patients with AI was 50% at 4 weeks as compared with 33.8% in the group with normal adrenal response. Steroid supplementation appeared to improve short-term survival when AI occurred, although these patients' overall mortality was worse than that of patients with septic shock and AAR. In conclusion, it is suggested that adrenal insufficiency should be suspected in patients with septic shock, who do not respond to conventional treatment.

THYROID HORMONES (T4, T3) AND TSH CHANGES WITH AGE IN NORMAL INDIVIDUAL

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Quantitative measurements of TT4, TT3 and TSH were analyzed in normal individuals of NWFP, Pakistan. The effects of age, gender, table salt types, sources of drinking water and locality where the subjects lived, on the levels of these hormones in the normal individuals after excluding those with evidence of any disease which could affect the thyroid functions were studied. The normal ranges for TT4, TT3 and TSH are 50- 170 nmol/L, 0.5- 2.9nmol/L and 0.2- 5.9mlu/L respectively. The sex, age, salt type, drinking water and areas of different strata of NWFP have the major influence on the TT4, but slightly affect the TT3 and TSH levels. The influence of age on TT4, TT3 and TSH levels is greater in the first and second decades of life in comparison with later stages of life. The variations present in the levels of hormones are due to the physiological adaptations of the body needs.

THYROID HORMONES AND TSH: QUANTITATIVE MEASUREMENTS IN NEONATES

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To evaluate the Thyroid hormones, essential for normal brain development and TSH in neonates, Radioimmunoassay measurements of serum concentrations of thyroid hormone [total thyroxine (TT4), total triiodothyronine (TT3), free T4 (FT4)] and Immunoradiometric measurement of serum thyroid stimulating hormones (TSH) were conducted in 80 full-term newborn infants between birth and 30 days of age. Of the total 80 neonates, 54 were males and 26 females. The ranges of TT4, FT4, TT3 and TSH found in this study were 40-200 nmol/L, 8-30nmol/L and 0.1-25µIU/ml respectively. TSH showed significant changes in both male and female neonates, having highest values in early days after birth and declining with age progression. TT4 level was slightly higher in early days after birth as compared to data taken after that. The levels of TT3 and FT4 show no significant changes. There was no effect on hormonal levels regarding gender. While the gestational age showed significant difference in hormonal levels. The levels were significantly higher in full term as compared to preterm neonates. The fluctuations in thyroid hormones and TSH are of transient nature as a response to environment and growing age may affect the growth of child.

CIRCULATING HCV GENOTYPES IN DISTRICT MARDAN NWFP

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The clinical outcomes of different patients infected with hepatitis C virus (HCV) ranging from acute resolving hepatitis to chronic liver disease consisting liver cirrhosis or HCC. Detection of the infecting virus genotype has become important in the exploration of many aspects of HCV infection, including epidemiology, pathogenesis and reaction to antiviral therapy. 147 individuals (121 HCV RNA positive and 26 Normal individuals) were included in this study. RNA was extracted from serum, reverse transcribed into cDNA and the core region of HCV genome was targeted by multiplex PCR. Of the HCV RNA positive individuals 88 were male and 33 were female. Genotype 3a was the most prevalent one of all the genotypes observed followed by 3b. 2a genotype was observed but 1a was also found in 10.89% individuals. 25.41% of the HCV RNA positive samples were not typed. 6.05% of patients were found having mixed genotypes. HCV Genotype/s was evaluated with patient gender but no significant correlation was found (Roy's Largest Root = 0.487, $P > 0.05$ NS). HCV type 3 and 2 are prevalent in this area which can better respond to interferon therapy but type 1 and 4 were also circulating which need longer therapy time period. Proper epidemiological studies, treatment strategies should be initiated and preventive measures should be taken to control the spread of this dreadful disease.

FLUCTUATION OF LIVER ENZYMES WITH CIRCULATING HCV GENOTYPES

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To find out the correlation of HCV genotypes with liver enzymes fluctuation/degree of liver damage in chronic HCV infected patients. A total of 84 blood samples from chronic HCV patients were collected from various hospitals and diagnostics labs of Peshawar district. Patients were analysed for HCV genotypes and Liver enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT and AST were performed once in a month (two readings for each patient) for six months. RNA was extracted; reverse transcribed into cDNA and was amplified by nested PCR. Type specific PCR was performed for genotyping and the product was resolved on a 2% agarose gel. ALT and AST level (monthly) for the entire period of six months were

correlated with HCV genotype, mixed genotypes and in samples which were not typed. ALT level fluctuation was analysed with specific HCV genotype/s of each individual and was found non significant (Student's t test = 1.84, $p > 0.05$ NS). AST level for the entire 6 month data was compared with the detected HCV genotype, which was also statistically found non significant (Student's t test = 6.43, $p > 0.05$ NS). In conclusion, our study shows that there is no apparent correlation of HCV genotype with liver enzymes fluctuation or the degree of liver injury in chronic HCV carriers. Other methods like liver histology should be followed to accurately assess the degree of liver damage in respect to HCV genotype and to decide therapeutic plan in patients chronically infected with HCV.

DNA TESTING AND GPS POSITIONING OF SNOWLEOPARD (*PANTHERA UNCIA*) GENETIC MATERIAL IN THE KHUNJERAB NATIONAL PARK (KNP) GILGIT-BALTISTAN

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Snow leopards (*Panthera uncia*) are elusive endangered carnivores found in remote mountain regions of central Asia. Reliable population estimates are necessary for effective conservation and management. Fecal genotyping has been used successfully to estimate the population size of several elusive mammalian carnivore species. We used noninvasive DNA sampling as a tool to estimate the population of snow leopard (*Panthera uncia*) in Karachani within KNP and Soqterabad Nallah outside of KNP. We successfully analyzed 24 amplified samples from Karachani nallah and 8 samples from Soqterabad nallah and their locations were determined by GPS positioning and recorded on existing map of the nallah. Samples s9, s14, s19 and s22 from Soqterabad produced the expected DNA fragments with three different banding patterns whereas sample s20 appears to show a different banding pattern from other. This could indicate that the sample s20 is from a different animal to the others whereas; in Karachani we could be able to identify two different banding patterns among the 24 amplified samples through DNA fingerprinting. Five samples k27, k33, k43, k52 and k63 out of 24 total amplified produced a typical banding while the 19 samples produced identical patterns, thus, we identified 2 unique DNA banding patterns from the 24 scats in our final data set that indicates the presence of 2 individuals in Karachani. Our result determine that at least one snow leopard was present in the area studied. The preliminary findings (DNA finger printings) indicate that there may be two individuals in Karachani and three individuals in Soqterabad Nallah. The study showed that non-invasive genetic sampling could be successfully carried out for snow leopard population estimation in Gilgit-Baltistan in combination with more established survey techniques and drawing on the expertise of trackers and wildlife experts in the field.

DECOLOURIZATION OF AZO DYE SYNAZOL RED 6BN BY FUNGI

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Azo dyes are widely used in textile, paper, leather, cosmetics, and food industries and are rather recalcitrant to conventional sewage treatment system. Since most of them have mutagenic or carcinogenic effects, industrial effluent containing azo dyes must be treated before it is discharged into the environment. A wide variety of microorganisms is present in industrial effluents carrying toxic chemicals and the biological processes by microorganisms have received considerable attention for the treatment of dye-containing wastewater. The present study deals with the isolation of azo dye synazol red 6BN degrading fungi from industrial wastewater. *Pleurotus ostreatus*, *Aspergillus niger*, and *Nigrospora* sp. were grown in salt medium containing synazol red 6BN and were able to decolorize 85%, 78% and 90% synazol red 6BN, respectively. The effect of temperature and pH on the decolorization of the azo dye was also investigated. The dye degradation ability of these fungi indicates that, these can be used for amelioration of azo dyes polluted industrial wastewaters.

RECENT OUTBREAKS OF DENGUE IN NORTHERN PAKISTAN ARE DUE TO RE-EMERGENCE OF *Aedes aegypti*

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Dengue is a mosquito-borne viral disease transmitted primarily by *Aedes aegypti* and secondarily by *Aedes albopictus*. Around 1930, *Ae. Aegypti* was found in most of Pakistan. By 1980 its distribution was limited to southeastern parts of Sindh Province. By 1983, it was found in Karachi City but not in any of the several localities surveyed from Hyderabad to Thatta. Thus there has been a remarkable reduction in the distribution of *Ae. aegypti* between 1930s and 1980s. The last record from northern Pakistan was from Kohat-Hangu Valley in 1949. Thereafter this mosquito was not found during extensive mosquito surveys in NWFP and Punjab till 1990s. It was for the first time in 1993 that we found this mosquito as a formidable pest, breeding in large numbers in tyres at Landi Kotal, where it was introduced by tyre importation. In 1995 we recorded it from Peshawar City. In 2003 it was found in Haripur Area. This year (2008) we found it in Abbottabad and Mansehra. Now it is widespread in northern Pakistan, and presumably throughout Pakistan. Eradication of *Ae. aegypti* from most, if not all, of Pakistan occurred after 1950s, coincidentally with the national malaria eradication program, a phenomenon

that may be called a collateral benefit. This mosquito has apparently re-emerged as a consequence of the cessation of malaria eradication program. There has been no epidemic of dengue as long as both dengue and its principal vector, *Ae aegypti* were endemic, and that situation prevailed up to 1950s. Thereafter, with the eradication of the vector, transmission of the disease ceased, resulting in the lack of herd immunity in human population. With the recent (over the past 10-15 years) re-emergence of the vector after about a generation time, the susceptible population is confronted with the risk of epidemics in several areas. The first ever recorded outbreak of dengue fever (DF) and dengue hemorrhagic fever (DHF) occurred in Karachi in 1994. In October 2003, there occurred an epidemic of dengue fever in a number of villages in Haripur and Attock districts. It was actually a regional epidemic as thousands of cases were reported from Delhi and surrounding areas. This year (2008) a large number of dengue cases were reported from Lahore and Rawalpindi Areas in Punjab and Haripur area in NWFP during the October-November season. Many more cases or outbreaks might have gone unnoticed. Widespread re-emergence of the vector explains the recent widespread outbreaks of dengue in Pakistan. We should get ready for more outbreaks in the coming years. An efficient surveillance system and high level of vigilance are needed at national, provincial and district levels. Control of the vector should be the corner stone of the strategy for prevention of dengue outbreaks. Genuinely trained vector biologist especially culicidologists should be consulted for devising appropriate, cost-effective and sustainable control measures, instead of wasting resources on the recommendations of some hand-picked poorly-trained and self-proclaimed 'experts' or 'advisors'.

PURIFICATION AND CHARACTERIZATION OF β -GLUCOSIDASE PRODUCED BY *TRICHODERMA KONINGII*

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An indigenous strain of *Trichoderma koningii* was used for purification and characterization of extracellular β -glucosidase after fermentation of 1.5 % wheat bran at 30 °C. β -Glucosidase was purified to homogeneity level by using a combination of $(\text{NH}_4)_2\text{SO}_4$ precipitation, gel filtration and ion-exchange chromatography. The purified enzyme possessed a molecular weight of 94 kDa, optimal pH of 5.5 and an optimal temperature of 55°C. Temperature- and pH-dependent kinetic parameters for β -glucosidase hydrolysis were determined. Enzyme exhibited stability up to 80 °C and over a pH range of 6.0-9.0. Purified enzyme showed a half-life of 90 min at 60° C. It exhibited Michaelis-Menten kinetics. We determined K_{cat} and K_m at optimum pH and

temperature. Magnitudes of activation energy, enthalpy, entropy and Gibbs free energy demand for active catalysis and inactivation were calculated. The enzyme is implicated to perform various functions in different organisms. Fungal and bacterial β -glucosidases have a very important role in conversion of biomass to ethanol by hydrolysis of cellobiose to glucose.

CORN SYRUP LIQUID AND MOLASSES INCREASE BIOMASS OF *METARHIZIUM ANISOPLIAE* STRAINS

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The addition of corn steep liquor (CSL) and sugarcane molasses (SM) in liquid growth medium for mass production of mycoinsecticide was investigated. Most virulent local isolate of *Metarhizium anisopliae* was for preliminary screening of growth media and then growth of several local isolates from termites was compared. Biomass of the fungus in growth media with corn steep liquor and sugarcane molasses was significantly increased (1.69 g) as compared to growth media without these two components (0.91 g). The presence of SM could not bring as much change in biomass (0.8 g) when compared to media with CSL (1.03 g). Both SM and CSL without any other material yielded significantly high biomass (1.17). Incorporation of CSL had significant effect on growth of the fungus.

STUDY OF BACTERIAL FLORA OF AIR SAMPLES FROM EDUCATIONAL INSTITUTIONS OF LAHORE AS POLLUTION INDICATOR

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The aim behind this work was to know the different types of bacteria present in air samples of different educational institutions of Lahore, Pakistan. Air samples were collected by exposing the media in sterilized petri plates in summer for five minutes, taken to the laboratory and used for microbiological examination. The isolated bacteria in order of their number were *Staphylococcus aureus*, *Micrococcus luteus*, *Shigella dysenteriae*, *Aerococcus viridans*, *Micrococcus roseus*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Staphylococcus epidermidis*, *Hafnia alvei*, *Proteus mirabilis*, *Enterobacter cloacae*, *Alcaligenes faecalis*, *Bacillus cereus*, *Proteus morganii*, *Klebsiella aerogenes*, *Escherichia coli*, *Pseudomonas putida* and *Streptococcus faecalis*. The number of bacteria was very high during the study period in Government Kulyat-ul-Binat Degree College Lahore, Soofia College of Science and Arts Lahore and Government Islamia

College Civil Lines Lahore. The isolates were harmless saprophytes and some pathogens which reveal the fact that congestion of population, heavy traffic and less vegetation in respective localities contributed much in producing such heavy load of bacterial pollution in these educational institutions but the main source may be the persons harboring these. These results endorse awareness to common people about the level of hygiene in respective areas.

FINGER NAILS AS BIOINDICATORS OF METAL EXPOSURE IN TRAFFIC PERSONALS

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Increased urbanization have tremendously increased the use of vehicles also ,which are major contributors of environmental metallic pollution and adversely affecting human beings .Especially traffic personals are at large risk which remain exposed off to this hazardous exposure. The primary objective of this study was to determine the metal level in finger nails as potential predictive index of the metal exposure in their work environment. In order to investigate the effects of vehicles exposure, finger nails were collected from the traffic personals belonging to district Sargodha having work experience of more than three years. This paper deals with the quantitative determination of Pb, and cd in the nail samples of traffic personals along with their respective controls. The levels of these metals assayed by atomic absorption spectroscopy were compared with controls. Significantly higher levels of these of these metals were observed in traffic personals. Pb mean value found in traffic personals vs. controls were 9.22 ± 2.05 and 1.95 ± 2.55 $\mu\text{g/g}$ while these were as 0.44 ± 0.36 and 0.01 ± 0.008 $\mu\text{g/g}$ for Cd levels among traffic personals and control ones respectively. It was concluded that finger nails are good indicators of metal exposure

ADVERSE EFFECTS OF LORAZEPAM ON PRE-NATAL DEVELOPMENT OF MICE

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Lorazepam (a Benzodiazepine) was tested for its embryotoxic effects on the developing mice. For this purpose different concentrations of Lorazepam were prepared by dissolving it in distilled water in such a way that each 0.1 ml of the solution contains desired concentration, which were 10.00, 15.00 and 20.00 I-Ig/g body weight. These

doses were administered orally to the pregnant mice on day 8 of gestation. The treated pregnant mice were anaesthetized on day 18 of gestation and fetuses were recovered. The resulting samples were subjected to morphological and morphometric analyses. Morphological studies of fetuses showed abnormalities including microcephaly, hydroencephaly, microphthalmia, open eyelids, micromelia of forelimb and hindlimb, hemorrhagic spots on head and back and intrauterine growth retardation. Morphometric studies of body weight, crown-rump length, brain size, forelimb size, hindlimb size and eye size of fetuses showed significant decrease against control. The abnormalities observed during this study were dose dependent. The study indicates that the concentrations of Lorazepam used are potentially dangerous to the developing mice fetuses. Therefore, it is recommended that this drug must be used with extreme care.

CHARACTERIZATION OF PROTEOLYTIC *BACILLUS* SPP. FROM ENVIRONMENT

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Microbial proteases dominate the worldwide enzyme market with two-third used in the detergent and leather industry. Production of enzyme for industrial use, isolation, characterization and optimization of new promising strains is a continues process through out the world. During this study fifty strains were isolated, purified and screened for proteases production from soil and water samples of different localities. Twenty isolates produced clear zone on skim milk agar plates were characterized and checked for temperature and pH tolerances. Optimum production conditions for alkaline protease were determined and enzyme was characterized. The selected proteolytic strain was spore forming, gram positive rod shaped, gave good growth at 47°C and pH 12. Maximum protease units were produced at 48 hours. This protease is suitable for use in detergent.

SPREAD OF EPIDEMICS DURING MONSOON SEASON- FACTORS OTHER THAN DRINKING WATER

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In rainy season there are a series of disease (Typhoid, Diarrhea, Gastro etc) which causes serious health effect on human beings and some time lead them to death. Commonly these are water born diseases but there are a number of other factors for the spread of these diseases. Water after boiling for 5 minutes is safe for drinking purpose but other factors are seriously noticeable. Normally it is seen and it has become a common motion that drinking water supplied by government. agencies / department is a major

cause of spread of diseases, which are epidemic in nature, but in fact, there are many other factors responsible for spread of diseases. In this, study others factors are pointed out and rout of spread of epidemics are discussed. Study was done by collecting samples of ice and drinking water samples from vendors and direct supply of drinking water. These samples were analysed for physiochemical parameters and microbiological tests. It was seen that all these samples collected from vendors were contaminated with E. Coli (Type of Coli form bacteria) which is responsible a number of diseases including gastro. Water samples collected from direct supply were fit for drinking purpose.

INFLUENCE OF *BACILLUS CEREUS*-SNB-4 AND *BACILLUS THURINGIENSIS*-SN-8 (ANTAGONIST) ON MILD STEEL CORROSION FOLLOWING TWO YEARS SOIL BURIED CONDITIONS.

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Long term effects of microbiologically influenced corrosion (MIC) of mild steel coupons under field simulated soil buried conditions were analysed following an exposure of 2 years. Highly corrosive *Bacillus cereus*-SNB-4, its non-corrosion antagonist *Bacillus thuringiensis*-SN-8 and the both microorganisms together were cultured in mild steel coupons (MSC) containing soils supplied with water and nutrient broth. After 2 years' exposure, MSC exposed to *B. cereus* had several folds higher values of average percent weight loss (APWL) and corrosion rates than control and the one exposed to the co-cultured conditions. Interestingly, the co-cultural exposed coupons had significantly lesser values of APWL and corrosion rates than the control MSC. MIC protective role of the bacterial antagonist can potentially be exploited involving or exclusive of biological corrosive agents.

ISOLATION OF H₂ PRODUCING PURPLE NON-SULFUR BACTERIA FROM VARIOUS WATER SAMPLES

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Anoxygenic photosynthesis is a predominant property of certain facultative bacteria that play an important role in primary productivity of different aquatic environments. Here we report isolation and cultivation of purple non sulfur phototropic bacteria from samples representing different aquatic environments. The inocula were incubated in the presence of light Pigmented growth ranging from dark red to brown

indicated the presence of purple non sulfur bacteria. Four bacterial isolates were cultivated in a medium (Brown, 2008) and assessed for their hydrogen production potential under illuminated conditions and varied temperatures. The purple non sulfur species produced mixture of gases (CO_2 , O_2 and H_2) in a nitrogen depleted environment. One of the bacterial isolate also grew in a medium containing potato starch as main carbon source. Thus the purple non-sulfur bacteria have potential of starch waste effluents' treatment with simultaneous production of biohydrogen, a clean future fuel.

POTENTIAL OF ALKALIPHILIC PROTEASE PRODUCING BACTERIUM FOR FORMULATING BIODETERGENT

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Alkaline proteases are important for detergent formulations. Alkaline protease producing bacteria were obtained from culture collection of lab and revived. Three alkalophiles were identified morphologically and biochemically as *Bacillus cereus*. The isolate designated FJ10 showed maximum growth at 50°C and pH 9 with 1% inoculum. The enzymes were characterized and yielded maximum activity at 50°C. The enzymes were also partially purified by ammonium sulfate precipitation and dialysis. Both crude and partially purified enzymes were found capable of de staining blood. Alkali and thermotolerant nature of the bacterial proteases render them industrial relevance.

EXPERIENCE WITH SWINE FLU IN LOCAL POPULATION

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The Swine flu 2009 is a global outbreak of new strain of influenza A H1N1 virus which is highly contagious disease of respiratory tract & has become a public health problem around the world. In Pakistan first case of swine flu was reported in August 2009 at Karachi since then its incidence is increasing and 10,600 deaths have been reported worldwide. Symptoms of swine flu are mild in more than 90% cases while only 5-7% of patients develop complications such as bilateral pneumonia, pain or pressure in chest, hypoxia and respiratory failure. Three types of influenza viruses (A,B,C) cause human flu. Among these, two also cause influenza also in swine. The new strain is thought to be result of reassortment of four strains of influenza A virus subtype H1N1 including one strain endemic in human, 2nd endemic in birds, 3rd and 4th endemic in pigs. The new reassorted strain has acquired two new capabilities: (1) Human to human

spread (2) Enhanced virulence. Mortality rate is 1-4% which can be reduced to less than 1% with proper treatment protocol. In the present study, we aim to evaluate the incidence of swine flu in our local population. Confirmed case is person with acute febrile respiratory illness with laboratory confirmed influenza A (H1N1) virus infection by RT PCR or viral culture. In this study, nasopharyngeal samples from the patients were sent to the reference laboratory, for confirmation of swine influenza H1N1. Fever, cough and sore throat were in 100% patients, headache and running nose in 83%, nausea fatigue and chills were encountered in 67% where as 50% have joint & muscle pain, shortness of breath etc and six patients develop pneumonia & hypoxia like complications. Among these six, three patients were expire; first on 18th day, second on 22nd day and third on 30th day after admission. Two patients including one female with five months pregnancy recovered from illness and were discharged from hospital after three weeks and sixth patient improved clinically and is off the vent. Multi system failure has; been observed including renal, hepatic and abnormal hematological findings. Early recognition of clinical sign symptoms and risk factor for swine flu influenza A are helpful and particularly important for patients having underlying chronic illness. Patients may develop complication within 24-48 hours, if appropriate treatment is not administrated. Close monitoring of young children under 5 years and adults above 65 years & patients with increased susceptibility to nosocomial infection may lessen case fatality. In this study, positive patients have the pre existing underlying disease & age range 22-56 years these results are identical with International studies reported higher incidence in 20-50 years age group. In our set up increasing frequency of swine flu influenza A is becoming an important public problem and great concern for the health officials. Clinician should include swine flu influenza A in the differential diagnosis of the patients with acute febrile respiratory illness & criteria for suspected cases of influenza. Treatment must be started within 24-48 hours after onset of symptoms to achieve maximum recovery. Patients having flu like symptoms must stay at home for 7-10 day, avoid contact to others, cover nose and mouth with tissue while coughing or sneezing also dispose tissue properly and wash hands frequently with soap.

PHYSIOLOGICAL RESPONSES OF CATTLE TO HEAT STRESS

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Pakistan lies in the Warm Climate Zones of the world and is subjected to extended periods of high ambient temperature and relative humidity. The objective of this article is to review the physiologic/adaptive responses of acclimated cattle to heat stress emphasizing its affects on nutrient acquisition and metabolism, acid-base chemistry, and hormonal balance of the body. Heat stress invokes a decrease in dry matter intake,

enhanced digestibility and reduced blood flow to the forestomach. The energy metabolism decreases while the water and electrolyte metabolism increases. A nycthermal pattern of acid-base balance is manifested by a high respiratory rate leading to respiratory alkalosis in the hot day time hours; and lower urine pH and greater urine ammonia concentrations in the cool night time hours. Hormones involved in thermal adaptations include, prolactin, growth hormone, thyroxine, glucocorticoids, mineralocorticoids, catecholamines and antidiuretic hormone. These are either involved with nutrient partitioning and homeorhesis or for homeostatic regulation, augmented by thermal stressor. All these adaptive physiologic responses are the same in *Bos taurus* and *Bos indicus* but are less pronounced in the later, making it a thermotolerant breed. Cholistani breed of cattle may be utilized as a model to study the physiologic responses augmented by heat stress both on molecular and endocrinological levels.

SECTION - II

PESTS AND PEST CONTROL

ECONOMIC IMPORTANCE AND DEVELOPMENT OF SPATIAL SAMPLING PLANS FOR EUROPEAN CORN BORER, *PYRAUSTA NUBILALIS* (LEPIDOPTERA: PYRAUSTIDAE)

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Sampling plans for adults, *pyrausta nubilalis* their spatial distributions were characterized and economics importance of sampling plans were evaluated by comparing sampling costs between spatial and conventional (non-spatial) sampling plans. Semivariogram modelling and spatial by with distance indices showed that European corn borer adults were significantly ($P < 0.05$) aggregated at peak population densities and any two samples were spatially correlated within approximately 45 m, with 39-90% of the variability explained by spatial dependence. Sampling costs for spatial sampling plans linearly increased as the sampling distance decreased and exponentially increased as the field size increased. Although sampling costs for non-spatial sampling plans were generally lower, spatial sampling plans could be more economical when the mean insect density became lower and the field size became smaller. This study demonstrated that spatial sampling plans could be optimized to minimize the sampling costs and maximize the spatial resolution.

COMPARATIVE SURVIVAL OF *CHRYSOPERLA CARNEA* (STEPHENS) (NEUROPTERA: CHRYSOPIDAE) IN COTTON WITH AND WITHOUT INSECTICIDES UNDER NATURAL FIELD CONDITIONS

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Cotton, *Gossypium hirsutum* L., the economy dependent crop in agricultural sector of Pakistan. Its yield for the last few years is reduced by the loss of crop due to

some factors and among them, the heavy pest attacks is the most important one. Insecticide is the common practice of insect pests control, which does not meant to be the factor of increased yield. Besides environmental pollution, these insecticides also cause the destruction of naturally occurring fauna of bio-control agents. *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is one of the most important natural predator of cotton pests and the repeated use of pesticides in crops disturbing its natural equilibrium. Present field study was therefore, planned to evaluate the natural presence of *C. carnea* in cotton and a field trial under natural conditions was conducted at Rehmat-ul-Allah Tareen farm in Lodhran in year 2007. Cotton cultivar MNH-786 was planted by making two treatments each with three replicates with plant to plant and row to row distance of 76 and 30 cm. In one treatment, pesticides were used according to the need of pest management of the crop, while in other treatment no pesticide was used and this treatment separated by a heavy strip of sorghum crop. Standard agronomic practices were adopted throughout the season. Data were collected on weekly basis from each replicate of both treatments by observing eggs of *C. carnea* from five randomly selected plants. Results showed that presence of *C. carnea* eggs on pesticide free treatment was comparatively higher to that of pesticide treatment. The overall presence of *C. carnea* eggs was highest in the month of August and September while lower in June and July in both the treatments.

**AGGREGATION PHEROMONE FOR IPM OF COLORADO POTATO BEETLE,
LEPTINOTARSA DECEMLINEATA (COLEOPTERA: CHRYSOMELIDAE) IN
ARDABEEL REGION, IRAN**

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The relative number of colonizing adult Colorado potato beetles *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) coming to pitfall traps baited with the aggregation pheromone (5')-3,7-dimethyl-2-oxooct- 6-ene-1,3-diol [(S)-I] and the use of the pheromone in a trap crop pest management strategy were evaluated in the field for the first time. More than five-fold more adult *L. decemlineata* were caught in pitfall traps baited with the pheromone compared with control. However, attraction to the pheromone diminished after 5 days in the field. In the trap crop management strategy, more colonizing adults were present in pheromone-treated rows of potatoes compared with untreated middle rows. Significantly fewer *L. decemlineata* egg masses and larvae were found in potato plots that were bordered by pheromone treated rows, or bordered by imidacloprid + pheromone-treated rows, or rows treated at-planting with imidacloprid

compared with untreated (control) potato plots. Densities of *L. decemlineata* egg masses and larvae and percentage defoliation were significantly lower, and marketable tuber yield were significantly higher, in conventional imidacloprid-treated potatoes compared with all other treatments. Although, our results demonstrated the potential for use of the aggregation pheromone in the management of *L. decemlineata* in the field. However, there is needed to optimize the release rates of the attractant and incorporate control methods for cohabiting pests.

**TO STUDY EFFECTS OF HETEROGENEOUS ENVIRONMENT ON THE
OVIPOSITION DECISIONS OF THE SEED BEETLE, *ACANTHOSCELIDES*
OBTECTUS (COLEOPTERA: BRUCHIDAE)**

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Acanthoscelides obtectus, distribute their eggs on various-sized seeds when the size of seed was varied during the egg-laying period. Beetles were allowed to lay eggs on one of three arrays of 64 Common beans. Each array contained four size classes of seed, ranging from small (5.0-5.5 mm diameter) to large (6.5-7.0 mm), but differed in how they were distributed within the environment. In the most heterogeneous condition (the 64-patch design), the four sizes were interspersed, while in the least heterogeneous condition (the four-patch design) they were grouped into four separate blocks. Thus, a beetle exploring the 64-patch design frequently encountered all four seed sizes, whereas a beetle exploring the four-patch design rarely encountered a change in bean size. Beetles experiencing greater seed size heterogeneity were more likely to lay eggs on larger seeds, whereas those in the blocked condition were more likely to oviposit on small seeds. Beetle responses to seed size heterogeneity suggested that the degree of preference for large seeds depends on a female. Female beetles exhibited size discrimination throughout their egg-laying process; however, there was a trade-off between seed size and egg discrimination (*i.e.* avoiding those seeds already containing developing eggs) in response to the change in fitness gained from either laying on larger seeds or lower egg-load seeds during the egg-laying process. Our model provides the first evidence that evolving seed size discrimination ability was adaptive for the seed beetle with egg-discrimination ability.

**TO STUDY REDUCTION IN EGG HATCH AFTER A SUBLETHAL DOSE OF
DIFLUBENZURON TO LARVAE OF THE CODLING MOTH, *LASPEYRESIA*
POMONELLA (LEPIDOPTERA: OLETHREUTIDEA)**

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The effects of a sublethal dose of diflubenzuron on egg hatching in *Laspeyresia pomonella* were examined under laboratory conditions. When LD₁₀ of diflubenzuron was topically applied to newly moulted fifth instar larvae of either sex, a significant reduction in both the number of eggs laid and subsequent hatching was observed after mating but no significant differences in daily of oviposition were observed when compared with the control. In addition, examination of the unhatched eggs revealed that the number of unfertilized eggs were greater than those that were fertilized but there were significantly more unfertilized eggs laid by treated insects. Interference by diflubenzuron, transferred by copulation through sperm fluids or ova, appeared improbable. All the fertilized unhatched eggs in the treated crosses died at an earlier stage than those of the control. In the female-treated crosses, the egg size was significantly reduced compared with the control or male-treated crosses. During mating, the treated-males transferred significantly lower-weight spermatophores into the females. The weight of spermatophores transferred by untreated males was the same to both treated and untreated females. The duration of mating was not affected by diflubenzuron treatment.

**POPULATION ECOLOGY (VERTICAL AND DIURNAL DISTRIBUTION) OF
TERMITES OF IRAN**

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The present study was based on a survey of termite fauna of Iran conducted during 2006-2008. During survey, four habitats were described. 1-Forest habitat: In forest habitat two species i.e., *Microcerotermes diversus* and *Anacanthotermes vegans*, were recorded foraging. Vertical distribution revealed significantly ($P < 0.05$) more termite population at 30 cm depth than at 15 cm or 45 cm depth. As regards diurnal distribution, total number of termites collected were 234, 200 and 163, respectively. Statistically, however the

variation in the number was non - significant ($p>0.05$). 2- Grassland habitat: In Grassland habitat only, *Microcerotermes diversus* was recorded foraging. Vertical distribution revealed significantly different ($p<0.05$) more termite population at 30 cm depth than at 15 cm depth. But, no correlation between atmospheric temperature or relative humidity and number of termites could be established. As regards diurnal distribution, total numbers of termites collected were 35, 53 and 24, respectively. The differences in number, however, was not significant ($P>0.05$). Lesser termite Population was recorded in grassland habitat as compared to that of forest habitat. Population density of *Microcerotermes diversus* varied from 00.0/m² to 7.20/m². 3- Desert habitat: In desert habitat, *Psammotermes Prohybostoma*, was recorded foraging in the desert habitat. Vertical distribution revealed more termite Population in deeper layer of 45cm. More soil moisture at deeper layer may be the source of attraction. Population of termite was 41, 69 and 38 respectively, but the differences were not significant ($p>0.05$). Population density in desert habitat varied from 0.61/m² to 10.08/m². 4- Cultivated habitat: In cultivated habitat a harvester termite, *Anacanthotermes vagans* was recorded foraging. It is worth noting that grassland, desert and cultivated habitat have different termite species, in cultivated habitat there was more population in upper 15 cm depth. The number of termite population was 22, 72 and 25 respectively, and the differences were not significant ($p>0.05$). Population density varied from 6.17/m² to 10.28/m². No linear relationship between the population and atmospheric temperature and relative humidity could be established. Termite diversity (studied by soil cores) on Simpson's Scale was 59%. Only three species of termites were recorded by soil cores. The value of H, H.max and E were 1.427, 1.5849 and 0.9003 respectively. As regards concentration of dominance, *Anacanthotermes vagans* with value of 0.26 was more dominant as compared to other species.

COMPARATIVE EFFICACY OF SOME INSECTICIDES AGAINST COTTON WHITEFLY, *BEMISIA TABACI* (HOMOPTERA: ALEYRODIDAE) UNDER FIELD CONDITIONS

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Cotton, *Gossypium hirsutum* L., is the most important cash crop of Pakistan, known as “white gold”. Pakistan is the world’s fourth largest producer of cotton and the third largest exporter to raw cotton yet facing the problem of low yield per acre. Many factors are contributing towards the low yield of cotton, among them the heavy insect pests attack are the significant which are mainly divided into two groups as sucking and

bollworms. Among the sucking pests, whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) is the most injurious pest, causing significant loss to cotton by sucking the cell sap of plant leaving the stunted growth and become the factor of low yield. The present study was therefore, planned to evaluate the comparative efficacy of five insecticides viz. acetamiprid, buprofezin, diafenthiuron, imidacloprid and thiamethoxam against whitefly and carried out at Khakwani farm Distt. Multan during 2008 by planting cotton cultivar MNH-786 under natural field conditions with standard agronomic practices. Pest population data was recorded before and after the application of each spray. Results showed that buprofezin was observed the most effective against whitefly nymphs population among the tested insecticides, while acetamiprid was recorded as the most effective against whitefly adults population followed by diafenthiuron and imidacloprid; whereas, thiamethoxam was found to be the least effective on both adults and nymphs population of whitefly.

**EVALUATION OF DIFFERENT CHEMICALS FOR THE ATTRACTION OF
MALE AND FEMALE FRUIT FLIES, *BACTROCERA ZONATA* (DIPTERA:
TEPHRITIDAE)**

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Among tephritid fruit flies, *Bactrocera zonata* (Diptera: Tephritidae) is a polyphagous and principally destructive pest of many fleshy fruits. Even though a male attracting system is existing for *B. zonata*, but its female attracting system is not available up till now. The aim of this field experiment was the evaluation of Torula Yeast, Boric Acid, Ammonium Acetate, Trimethyl amine and Putredne in different concentrations and combinations to develop a female attracting system for *B. zonata*. The chemicals were tested on cotton wicks placed inside a plastic traps separately. The flies catch were recorded daily and sexed. Studies indicated that the attraction of female fruit flies in Ammonium Acetate, Trimethyl amine and Putrecine compounds was significantly higher than the males. Response of both sexes to Torula Yeast, Boric Acid increased progressively with the increase in concentrations and significantly higher flies were caught in Torula Yeast and Boric Acid in a ratio of 7:3 respectively. Findings suggested that both sexes of *B. zonata* consistently exhibited a significant positive response to odor of proteinaceous food attractants. These results demonstrated the potential of locally produced bait as a cheaper alternative in fruit fly control through annihilation technique.

DETERMINATION OF PESTICIDE RESIDUES IN SOIL OF NAWABSHAH DISTRICT SINDH, PAKISTAN

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Pesticides residues organophosphate (OP), pyrethroids and organochlorine (OC) classes were monitored in soil samples collected from cotton growing areas in Sindh. The Gas Chromatography (GC) was employed for analysis, equipped with Electron Capture Detector (ECD) using the capillary column followed by the extraction of pesticides from soil samples. 27 soil samples were monitored of dichlorvos, fenvalerate, dimethoate, methyl parathion, fenitrothion, cypermethrin, endosulfan, deltamethrin, mevinphos, chlorpyrifos, profenofos and dicofol pesticides. All the soil samples analyzed were found contaminated with tested pesticides and the varying degree of concentration and frequency found in the top soil. The most widely detected pesticide was chlorpyrifos and found in 16 sample with mean concentration of 0.486 mg kg^{-1} . Endosulfan was the second most often detected pesticide investigated in 15 samples containing the mean concentrations of 0.426 mg kg^{-1} . Dimethoate was the third most detected pesticide in 14 samples with mean concentration 0.555 mg kg^{-1} . The pesticides accumulated in the soil for relatively longer period of time and then passed into various parts of the plant grown on the contaminated soil. It is concluded that to prevent adverse effects on public it is a must to carryout regular monitoring system and to establish control measures.

EFFECTS OF DIFFERENT PESTICIDES TO LARVAE OF GRAM POD BORER *HELICOVERPA ARMIGERA* (HELIAR) USED IN CHICKPEA UNDER THE FIELD AND LABORATORY CONDITIONS

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During the last decade ample emphasis has been placed on reduced use of synthetic pesticides, and the use of bio-pesticides is encouraged. Biological insecticide Helicovex based on *Helicoverpa armigera* nucleopolyhedrovirus (HearNVP 7.5 X 1012 PIB/ L) was tested for the control of the larvae of *H. armigera* (Heliar) in comparison with Endosulfan 35 EC in chickpea. Observations on the incidence of pest were recorded

by counting the larval population, pod infestation (calculated as percent damage) for *H. armigera*, and grain yield. The larvae ingest the virus become infected, ultimately killed by virus applied at the dosage of 200 ml per hectare. Among the tested chemicals, under field environment, Helicovex was the most toxic insecticide to the larvae at the recommended rate and classified as highly harmful and statistically at par with Endosulfan. In another set of experiment under laboratory conditions, Helicovex had shown 100 % potential for pest's mortality than synthetic pesticide in comparison to the field environment.

IMPLICATING CONSERVATION OF PARASITOID *TRICHOGRAMMA CHILONIS* (TRICHOGRAMMATIDAE: HYMENOPTERA) THROUGH SUPPLEMENTATION OF FOOD ATTRACTANTS IN RICE FIELD

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This experiment comprised treating of rice crop to control borers with biocontrol agent *Trichogramma chilonis* reared on the eggs of angoumois grain moth *Sitotroga cerealella*, in conjunction with different food sprays as a diet for the released natural enemy. The treatments were supplementing *T. chilonis* with concentrated 5 % solutions of sugar and protein hydrolysate sprayed separately, third treatment consisted of treating crop with bio-control agent and sprayed with 1: 1 ratio of protein hydrolysate and sugar solution. Within fourth treatment rice was treated with bio-control agent and no supplemented food was used for *T. chilonis*. The establishment or conservation of the *Trichogramma chilonis* was monitored by placing fresh eggs of *S. cerealella* in the field exposed for 24 hours, and then recorded the parasitoid emergence. Results exposed that the parasitism in all the treatments was low during early crop growing season and increased gradually near harvest. The mean parasitization percentage of *S. cerealella* eggs by *T. chilonis* displayed that increased parasitoid densities were observed in all the treatments, where food was supplemented for conservation purpose. Nevertheless, the parasitism was elevated in the crop treated with bio-control agents and sprayed with both protein hydrolysate and sugar in combination. Accordingly, maximum grain yield was obtained from minimum percent infestation level of borers due to plants treated with biocontrol agent along with supplementation of sugar and protein hydrolysate solutions as food attractants.

**SUSCEPTIBILITY OF THE FRUIT FLY, *BACTROCERA ZONATA*
(SAUNDERS)(DIPTERA:TEPHRITIDAE) AGAINST SOME INSECTICIDES
UNDER LABORATORY CONDITIONS**

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Fruit Flies rank among the world most serious pest of horticultural crops. They not only cause direct losses in the yield but also retard international trade in fruits and vegetables. The estimated losses annually due to fruit flies attack about \$200 million in Pakistan. There are about 11 species of fruit flies in Pakistan, among them *Bactrocera zonata* (Saunders), is one of the most serious polyphagous insect pest. It attacks a wide range of fruits and vegetables hosts, such as mango, peach, fig, guava, citrus, tomato and apple. Amongst control measures of fruit flies chemicals are the most effective one. In chemical control of fruit fly insecticides are used in different forms such as bating, with attractants and cover sprays. Present study was therefore carried out to estimate the susceptibility of *B. zonata* against 10 insecticides *i.e.*, profenofos, indoxacarb, spinosad, bifenthrin, lamdacyhalothrin, malathion, deltamethrin, trichlorfon, emamectinbenzoate and imidachloprid. *B. zonata*, larvae were collected from infested mango fruits from 5 different localities of Multan and their culture were maintained in laboratory at $27 \pm 1^\circ\text{C}$ and R.H.60 \pm 5%.The adult flies were fed artificial diet. Different concentrations of insecticides were applied in 1 μ l droplet on the pronotum of 3 to 5 days old fruit flies with the help of Burkard micro applicator. The results showed that the culture of fruit flies collected from different locations were highly susceptible to malathion and tolerant to emamectinbenzoate followed by trichlorfon, as compared with laboratory maintained susceptible fruit flies culture.

**COMPARATIVE EFFICACY OF SOME INSECTICIDES BEARING NOVEL
MODES OF ACTION AGAINST FIELD STRAINS OF *TRIBOLIUM*
CASTANEUM (HERBST.) IN SOUTHERN PUNJAB**

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Some new chemistry insecticides bearing novel modes of action were tested against adults of *T. castaneum* collected from southern region of Punjab. Results revealed

that Emamectin 1.9EC was most toxic of all followed by Abamectin 1.8EC, Lufenuron 0.50 EC, Spinosad 240SC and Indoxacarb150SC when treated for 48 hours. D.G. Khan strain was most susceptible against Emamectin while Layyah strain was most resistant with LC₅₀ values of 0.07 ppm and 0.31ppm respectively. Multan strain proved least susceptible towards Abamectin while D.G. Khan remained resistant by showing 3.59ppm and 11.4 ppm LC₅₀ values, respectively. In case of Leufenuron, Khanewal strain was susceptible of all and Multan strain was most resistant with LC₅₀ of 237ppm and 968ppm respectively. Muzaffar garh is most susceptible while Multan strain proved most resistant against spinosad showing LC₅₀ values of 128ppm and 1284ppm, respectively. Multan strain proved most susceptible against Indoxacarb as compared to other strains while Layyah strain was most resistant with LC₅₀ values of 2140ppm and 3853ppm, respectively.

**THE PROPENSITY OF DIFFERENT LARVAL STAGES OF LACEWING
CHRYSOPELTA CARNEA (STEPHENS) (NEUROPTERA: CHRYSOPIDAE) TO
CONTROL APHID EVALUATED ON CANOLA *BRASSICA NAPUS* L.**

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The aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae), is viewed as a pest principally on oilseed rapes, which can attain very high densities on young plant tissue, causing water stress, wilting and reduced growth rate of the plant. Green lacewings are considered to be one of the most effective predators of aphids. Therefore, the use of chrysopid *Chrysoperla carnea* (Stephens) 1st and 2nd instars, and 3rd and 4th instars larvae were investigated under field conditions in *Brassica napus* L., variety Rainbow. Results indicated that predator, irrespective of their, developmental stage, reacted more positively for predation to the prey except untreated control. Of the different larval stages tested, the applications of 1st and 2nd instars larvae of *C. carnea* were more effective in reducing aphid population and increasing grain field compared with 3rd and 4th instars. In the similar fashion the releases of 3rd and 4th instars larvae of *C. carnea* were more effective in reducing pest and increasing yield compared with check treatment. Poor performance of 3rd and 4th instars larvae than inoculation with 1st and 2nd instars of *C. carnea* were due to their too late predation activities to play a direct beneficial role in crop protection.

**PROVISION OF SUPPLEMENTAL FOOD FOR THE CONSERVATION OF
BENEFICIAL INSECTS IN COTTON FIELD**

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The honeydews, nectars and pollens are the major source of food for the adult insects in the field. Different chemicals and other stimuli related to the insect food and their habitat were studied for the augmentation and conservation of natural enemies in the cotton field. For this purpose protein in the form of protein hydrolysate and carbohydrates in the form of sugar were sprayed alone as well as in combinations in the field. All the chemicals proved useful to enhance the population of beneficial insects including; *Chrysoperla carnea*, *Trichogramma chilonis* and *Grius* spp in the cotton field. Combined spray of protein hydrolysate and sugar significantly enhanced the populations of *C. carnea* and *T. chilonis* as compared to other treatments, where protein hydrolysate and sugar were used separately. However, the population of *Grius* spp was high in the treatment, where only sugar solution was sprayed as food supplement. Provision of such food supplements in the cotton showed the potential to increase the population of beneficial insects and subsequently the predation/ parasitism percentage for the successful management of cotton pests in the field.

**SIGNIFICANCE OF RELEASING PLACE AND TIME ON THE
ESTABLISHMENT OF PARASITOID, *TRICHOGRAMMA CHILONIS* IN SUGAR
CANE FIELD**

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Nine different sugar cane plots were selected at farmer's field near Tando Jam. *Trichogramma* cards obtained from the mass rearing laboratory were placed on different parts of the sugarcane plants at different times. The experiment was started from the month of February and continued till the end of October. Establishment of the parasitoids were monitored by placing fresh irradiated Angoumois grain moth eggs for 24 hours and brought to the laboratory to record the parasitism and emergence rates exposed in from the different plots. Studies revealed that crop microhabitat affected the parasitoid emergence. All biological parameters were significantly higher when the cards were placed at middle portion of sugar cane plant during evening hours. While, higher damage on emergence of the parasitoids by the secondary predators was recorded when the parasitoids were released by placing them at the lower portion of the plant. Temperature

also played a significant role on the establishment of parasitoids and peak numbers of the parasitoids were observed during the months of April and September when the mean temperature in the field ranged between 31 to 36°C.

PRODUCTION OF GOOD QUALITY PARASITOID, *TRICHOGRAMMA CHILONIS* (ISHII) FOR FIELD RELEASES

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Studies were carried out to determine the suitable ingredients in the adult diets of *Trichogramma chilonis* for production of good quality parasitoids. Mature pupae of *T. chilonis* were collected from the culture reared on the factitious host, *Sitotroga cerealella* and kept in small glass bottles separately till adult emergence. The adults were sexed and 5 pairs confined in different glass jars. Each glass jar having *T. chilonis* adults were provided with different ingredients, such as honey, sugar, protein as adult food separately in 10 percent concentration. Fresh Angoumois grain moths eggs glued on paper cards at the rate of 100 eggs per card were exposed for 24 hours in the oviposition cages on daily basis till death of the adults. Results indicated that sugar solution proved the best to enhance the fecundity and fertility of the parasitoid. The parasitism and adult emergence of the parasitoids were also higher when they were fed on sugar solution followed by the provision of honey solution. Feeding of protein solution proved significantly inferior among the food materials tested. No change in the sex ratio was observed when the adults were fed on different ingredients.

ACTIVITY AND BIOLOGICAL EFFECT OF *NEPETA CLARKEI* HOOK. F. (LABIATAE) AGAINST INSECTS OF MEDICAL AND VETERINARY IMPORTANCE

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To assess the larvicidal efficacy of a *Nepeta clarkei* Hook. f. (Labiatae) aqueous extract on *Culex* (Diptera/Culicidae), larvae. Fourth instars larvae were exposed as a

normal media supplemented with *Nepeta clarkei* formulations in different concentration (0.025%, 0.05%, 0.1 %, 0.15%, and 0.2%). A control group of larvae was exposed to an aquatic media without extract, reported nonsignificant mortality. The lethal concentration at which 50% of larvae failed to become pupate and died was found to be 0.1% at 24 hours of exposure. Significant reductions on growth and pupation, besides prolonged larval periods, were observed in treated and compared with control. The chloroform extract showed 100% mortality when applied for solvent toxicity in contrast, We produced no growth disruption within the tested range of concentrations.

EVALUATION OF IMMEDIATE IMPACT OF COMMONLY PRACTICED INSECTICIDES ON MORTALITY RATE OF COTTON SPIDER GUILD

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During 1998 and 1999 a study in three cotton fields was done in order to examine immediate impact of ten commonly practiced insecticides on spider guild for IPM, which significantly reduced spider densities. 589 spiders comprising, 222 adult and 367 young forming 59.0% of total catch. Eight families, 18 genera, 31 species in pitfall, six families, 11 genera and 18 species in jarring were recorded before pre- IA. On the whole 11 families, 35 genera and 80 species. Young of 44 ground and of 30 foliar species were present in pre-IA. ANOVA, DMR and stepwise regression indicated a serious reduction in spider population. *Pardosa oakleyi* (n=80) found to be the most common ground species forming (12.73%) was recorded after 24 and 72 hrs of IA, its population gradually decreased after IA but young were present in pre-IA" post- IA₁ and post- IA₂ except post- IA₁ of August and September. *Thomisus italongus* (n=22) was second in order of dominance most common foliar species whose population gradually decreased. Maximum young belonged to *Neoscona mokerji*. *P. sumatrana* and *Oxyopes rufisturnus* (n=27) showed their presence in all the sampling months in comparatively less number in pre-IA, post- IA₁ and post- IA₂, former was least affected by IA and its population increased between post-IA₁ and post- IA₂ except in post- IA₁ of August; latter was not present in pre-IA of August and post- IA₂ of September. *Gesa subarmata* (n=14) was found more resistant as compared to other species as its population remained unchanged or a little bit increased immediately after post-IAI. All other dominant species were totally absent in one or two months after post - IA₁ and IA₂. Average trapping and jarring diversities during post-IA₁ were (2.31, 2.04) in August, evenness was in post-IA₂ (0.76, 0.89) in September, richness was in pre-IA, (11.34, 14.66) in July, respectively.

**CODLING MOTH *CYDIA POMONELLA* (L.) MANAGEMENT USING
CHEMICAL TREATMENTS: MASS-TRAPPING IN RELATION TO DEGREE
DAYS IN UPLAND BALOCHISTAN**

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Codling moth *Cydia pomonella* (L.) is a key tortricid orchard pest in apple growing areas of Balochistan. Field trails were conducted in an apple orchard at Saria, Quetta during 1998 and 1999 to determine numbers of spray to kill maximum larvae of *C. pomonella* and to evaluate the field efficacy of insecticide Lorsban (Chlorpyrifos) 40 EC, Match (Livefenuran) 50 EC and Talstar (Biphenethrin) 10 EC against this pest. The experiments were laid out in a split plot design of four treatments including one control (check) replicated five times and in a Randomized Complete Block Design (RCBD) with six replicates consisted of four treatments plus a check included. Pheromone traps were used to monitor the emergence of moth and to examine the optimal time of spray application to control this insect. Results of insecticides revealed that Talstar (Biphenethrin) 10 EC was proved effective compared with Larsban (Chlorpyrifos) 40 EC, and Match (Livefenuran) 50 EC. The analyzed data on fruit infestation revealed highly significant differences between generations and treatments at P-value <0.001 in 1998 and 1999. Results of pheromone traps indicated that during 1998 the emergence of first and second generation moth was began at 97.04 and 663.04 degree days (DD) base 10°C, respectively while during 1999 first moth of the subsequent two generations was emerged at 0 97.66 and 707.93 DD.

**BIOLOGICAL SCREENING OF HILL TOON, *CEDRELA SERRATA* ROYLE
AND ITS TOXICITY AGAINST RED FLOUR BEETLE, *TRIBOLIUM
CASTANEUM* (HERBST)**

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Plant-derived substances have shown "insecticidal, synergistic and pharmacological effects. The present studies were conducted to evaluate the toxicity and effects of leaves extract of hill toon, *Cedrela serrata* Royte against red flour beetle, *Tribolium castaneum* (Herbst). Insects were reared under controlled temperature (30±2°C) and relative humidity 60±1%. Methanolic extract of *C. serrata* and their fractions using solvents of different polarities were also tested for antioxidant, phytochemical and spectrophotometric analysis. Antioxidant assay includes DNA protection assay and free radical scavenging activity using DPPH (2, 2-diphenyl-1-picryl-

hydrazyl). Toxicity and residual effects range NBF >CME (crude methanolic extract) >EAF (ethyl acetate fraction) >AQF, against *T. castaneum*. DNA protection assay shows that plant is safer to use because it has no damaging effects on DNA and in very small quantity when applied protected the supercoiled as well as open circular form of DNA. Conversion of DPPH to 2, 2-diphenyl-1-picryl-hydrazine by electron transfer method shows plant has significant antioxidant activity to scavenge free radical. NBF (n-butanol fraction) and AQF (aqueous fraction) show maximum ($IC_{50} < 1.0$ ppm) and minimum (IC_{50} : 5.6 ppm) antioxidant activity, respectively. Phytochemical analysis of these extracts indicates strong presence of alkaloids, reducing sugar, flavonoids, saponins, terpenoids, steroids, tannins, cardiac glycosides. Spectrophotometric analysis confirms the purity of fractions from crude extracts. Extracts of *C. serrata* show significant insecticidal and pharmacological activities.

DETECTION OF RESISTANCE IN KHAPRA BEETLE, *TROGODERMA GRANARIUM* AGAINST SELECTED INSECTICIDES FROM SOUTHERN PUNJAB, PAKISTAN

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Khapra beetle, *Trogoderma granarium*, Evarts is a notorious pest of stored products throughout the world including Pakistan. It is one of the major threats to stored wheat in Pakistan due to its high infestation potential. The major tools for its management are the two fumigants like methyl bromide and phosphine. There are number of reports about the resistance development against these fumigants from the subcontinent region. So the alternate for the management of this pest is the use of insecticides. We collected *T. granarium* from four different regions of Southern Punjab i.e., Multan, Khanewal, Muzaffar Garh and Dera Ghazi Khan. Four insecticides i.e., lambda-cyhalothrin, lufenuron, emamectin benzoate and spinosad were evaluated for the 5th instar larvae of *T. granarium* using standard residual film method against stored grain pests at 30 ± 5 °C and RH $45 \pm 5\%$. The LC_{50} values of each insecticide for all the four strains showed overlapping at 95 % confidence interval. The non significant differences were also observed when compared with 6th generation laboratory reared strain. These results revealed that these were equally toxic to larvae of all the strains of *T. granarium*. The most toxic insecticide was emamectin benzoate followed by lambda cyhalothrin and spinosad while lufenuron was the least toxic among the tested insecticides. Our studies suggest that these insecticides are the effective management tool for *T. granarium* to overcome the fumigant resistance problem.

SCREENING OF SOME INSECTICIDES AGAINST ISOLINES OF 2ND INSTAR LARVAE OF LEAFWORM, *SPODOPTERA EXIGUA* USING LEAF DIP METHOD

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Spodoptera exigua is one of the most destructive cosmopolitopn pests of agronomic cropss and vegetables. Screening of some of the insecticides used tridationally against *Spodoptera exigua* was done. Five insecticides (3 IGRs and 2 Pyrithriods), *i.e.* spinosad, emamactin, lufenuron , deltamethrin and chlorpyrifos were tested on recommended field doses. Standard leave dip method was used to test the insecticides on five different isolines of *Spodoptera exigua*. the results showed that there was no significant difference in relation to the toxicity of IGRs, however chlorpyrifos showed some level of resistance with LC50 value of 89.4 and FL value of 55.0-134 at 95% and the value of LC90 and FL value of 280-1224 at 95%. The insecticides recommended for the field applications against *Spodoptera exigua* showed very feeble resistance against tested insecticides and can be used for the control of *Spodoptera exigua* as per recommended dose.

ENZYMES AS A PREDICTOR OF INSECTICIDE RESISTANCE IN *CHRYSOPERLA CARNEA* (STEPHENS) LARVAE

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Insecticides from five different groups *viz.* carbamate (methomyl and carbosulfan), IGRS's (lufenuron), oxadiazine (indoxacarb), pyrethroids (bifenthrin, fenpropathrin, cyfluthrin and cyhalothrin) and microbial (spinosad and emmamectin benzoate) were applied onto the cotton, *Gossypium hirsutum* leaves by leaf dip method to investigate their effect on the enzymatic levels of *Chrysoperla carnea* after 48 hrs of treatment. Levels of acetyl cholinesterase and lactose de-hydrogenase were recorded post treatment of insecticides. Lufenuron and carbosulfan showed high levels of acetyl cholinesterase in 1st instar larvae. In contrast to this 2nd and 3rd instar larvae treated with indoxacarb attained highest levels of acetyl cholinesterase. The levels attained after insecticides treatment were almost equal to that of control. On the other hand maximum level of lactose de-hydrogenase were recorded in 1st instar larvae treated with spinosad and bifenthrin. In contrast to this indoxacarb and cyfluthrin produced high level of lactose

de-hydrogenase in 2nd instar larvae. Whereas 3rd instar larvae treated with bifenthrin and spinosad showed higher levels of enzyme. Both enzymes were significantly lower than the control. It was suggested that the levels of acetyl cholinesterase and lactose de-hydrogenase at larval stages can be considered as a predictor of insecticide resistance.

INTEGRATED MANAGEMENT OF MANGO FRUIT FLIES IN MANGO ORCHARDS

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Combined effect of three IPM techniques *i.e.*, male annihilation technique (MAT), bait application technique (BAT) and sanitation technique was measured to control fruit fly (*Bactocera zonata*) (Tephritidae: Diptera) in mango orchards during 2007 and 2008. Six experimental plots each of 140 acres were selected at different locations in Multan district. Six farmer's / control plots were also marked to record the natural infestation by fruit flies. Significantly lower rate of fruit fly infestation was recorded in experimental plots than their infestation in farmer's plot. It was concluded that these techniques can be used to reduce the fruit fly plague in mango gardens.

EFFECTS OF HIGH-TEMPERATURE FLUCTUATIONS ON APHID-PARASITOID COMMUNITY

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The effects of the frequency and severity of high-temperature perturbation were tested on a community consisting of aphids, parasitoids and pepper plants, green peach aphids and two parasitoids: *Aphelinus abdominalis* and *Aphidius matricariae*. Mesocosm communities were exposed to either daily or twice-weekly perturbations in temperature, with mid-day peaks at either 30 or 40 °C. Population growth in the aphid in both the presence and absence of parasitoids was affected primarily by the frequency of high-temperature perturbation, and was found slower in the high-frequency treatments. The two parasitoid communities were differentially affected by the frequency of high-temperature perturbation.

BIOLOGICALLY BASED PEST MANAGEMENT OF CORN

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Efficiency of *Chrysoperla carnea* (Stephens) (Neuroptera:Chrysopidae) and *Trichogramma chilonis* Ishaii (Hymenoptera:Trichogrammatidae) was studied with insecticides applied as seed treatment (imidacloprid @ 19g / 700g seeds), furrow (furadon @ 10kg / acre) and foliar (lambda cyhalothrin @ 330 ml / acre). *T. chilonis* Ishii was released in the form of ready to emerge ticho-cards. *C. carnea* was released in the form of stalked eggs on the paper strips. Minimum number of aphids (*Rhopalosiphum maidis*) (Fitch) (Homoptera: aphididae) were recorded in plots where lambda cyhalothrin was applied as foliar spray. Percentage damage done by maize stem borer (*Chilo partellus*) (Swinhoe) (Lepidoptera: Pyralidae) and sorghum shoot fly (*Atherigona soccata*) (Rondani) (Diptera: Muscidae) was significantly lower in plots having seed treatment combined with *T. chilonis* and with both *T. chilonis*, and *C. carnea* respectively. It was assumed that efficiency of biocontrol agents to manage corn pests may be increased when applied in combination with insecticides, mostly when insecticides are used for seed treatment; and that further research is needed to explore the benefits to use bio-control agents in combination with insecticides for environmentally safe management of corn-insect pest complex.

STUDY OF BEHAVIOURAL RESPONSE OF WORKERS OF A TERMITE *HETEROTERMES INDICOLA* TO DIFFERENT PLANT EXTRACTS

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The experiment was conducted to record the behavioural response of termite specie (*Heterotermes indicola*) against the plant extracts of Garlic (*Allium sativum*), turmeric (*Curcuma longa*), tea (*Carnellia sinensis*), green chilies (*Capsicum solanaceae*) and ginger (*Zingiber officinale*). Behavioral response including total distance, average speed and pause time by single termite worker was recorded by using Abid's track move software. The total distance covered by termite worker was minimum in turmeric *i.e.* 40% less than normal worker and in ginger it was maximum *i.e.* 5% less than a normal worker at 0 minute after the release. After 5 minute interval distance traveled in all the treatments decreased even more but in turmeric it increased and reached up to maximum after 10 minutes of release. In case of average velocity the same trend was observed in all the treatment except turmeric where average velocity was very low at 0 minute interval but after 10 minutes it increased up to 35% more than a normal worker. The maximum

pause time was observed in garlic after 5 minute interval and minimum pause time was observed in case of tea at all the three intervals *i.e.* after 0 minute, after 5 minutes and after 10 minutes. It was concluded that termite worker acted differently on the application of different toxic plants. The most unusual behaviour was observed in case of turmeric where mobility was very slow at 0 minute but after 10 minutes it reached to maximum.

POTENTIAL OF CITRUS SEED EXTRACTS AGAINST *Aedes albopictus* (SKUSE) (CULICIDAE: DIPTERA) LARVAE

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Temperature and more urbanization have led to issues like improper disposal of wastes, and breeding of pests. Every year huge amount of money is spent on the pesticides for the treatment of these waste yards and temporary water catchments. Still there are cases where organochlorinated hydrocarbons are widely being used. Plant extracts generally have essential oils with insecticidal activity. Such activities from Rutaceae particularly citrus have attracted greater concern due to their growth inhibiting effects against insect pests. Therefore the extracts from citrus varieties with insecticidal qualities were used against immatures of *Aedes albopictus* (Skuse). The results have revealed that rough lemon *Citrus jambhiri* and lemon *Citrus limon* had the lowest LC₅₀ values (119.99 and 137.26 ppm respectively), after 24 hours of exposure, followed by red blood *Citrus sinensis* (295.63 ppm), chakutra *Citrus grandis* (334.87 ppm), galgal *Citrus pseudolimon* (644.25 ppm), brazilian sour *Citrus aurantium* (905.96 ppm) and kinnow *Citrus reticulate* (1022.67 ppm). Narangi *Citrus mitis* had the highest LC₅₀ value (2069.117 ppm) after 24h of exposure followed by grape fruit *Citrus paradise* (1598.15 ppm) and musambi *Citrus sinensis* (1389.16 ppm), implying their least effectiveness against *Aedes albopictus* larvae. Percent mortality of *Aedes albopictus* larvae after 24h of exposure under lab conditions showed that lemon and rough lemon gave the highest percent mortality (88.9 and 95.6% respectively). Narangi (11.7%) showed the least percent mortality. After 48h, rough lemon and lemon gave almost 100% mortality (99.6 and 95.6% respectively). There was also a statistically significant difference among different citrus seed extracts in relation to time to kill. Rough lemon (LT₅₀, 2.51h) was the fastest killer followed by lemon (LT₅₀, 4.91h) and red blood (LT₅₀, 8.52h). Galgal showed 50% mortality after 26h; kinnow and musambi took almost two days (50.3 and 52.7h respectively) to give 50% mortality. Grape fruit took almost three days to cause 50% mortality. So it is clear that rough lemon, lemon and red bloods are the best varieties in terms of LC₅₀, % mortality and LT₅₀ while narangi is the least effective variety in terms of LC₅₀ and % mortality. Our studies, therefore, suggest that citrus seeds have essential oils which have greater potential in the management of *Aedes albopictus*.

ISOLATION OF FUNGI FROM VARIOUS LIFE STAGES OF MANGO BARK BEETLE, *HYPOCRYPHALUS MANGIFERAE* (COLEOPTERA: SCOLYTIDAE): A VECTOR OF MANGO SUDDEN DEATH DISEASE

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The mango bark beetle, *Hypocryphalus mangiferae*, has been reported as vector of mango sudden death disease in various countries including Pakistan and various fungi has been isolated from adult bark beetle only. The main objective of this study was to isolate mango sudden death fungi from life stages of bark beetle, *H. mangiferae*. For this purpose, three sites of mango orchards (Khanewal road, Nawab Pur and Band Bosan) were selected in the vicinity of district Multan. Different life stages of bark beetles were collected from diseased trees and fungal isolations were made individually from each stage as well as from different body parts (head, thorax and abdomen) of adult bark beetle. Four types of pathogenic fungi (*Ceratocystis fimbriata*, *Lesiodiplodia theobromae*, *Fusarium* sp. and *Phomopsis* sp.) were isolated from the sampled bark beetles. The maximum frequency of pathogenic fungus, *C. fimbriata* was isolated from adults (4.39 %) followed by grubs (2.22 %), eggs (1.66 %) and pupae (1.11 %) while the *Phomopsis* sp. was only isolated from adults (5.44 %). Mean frequency of *C. fimbriata* Isolated from abdomen and head of adult bark beetle was 4.40 % and 0.95 % respectively. While, *Fusarium* sp. was also isolated to its highest mean frequency from abdomen (8.69 %) followed by head (2.85 %). It is suggested that the consistent association of mango sudden death fungi with almost all life stages of *H. mangiferae* may help its dissemination in mango orchards.

PREDATORY POTENTIAL OF *CHRYSOPERLA CARNEA* (STEPHENS) (NEUROPTERA: CHRYSOPIDAE) AGAINST APHID IN TOMATO UNDER FIELD CONDITIONS

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The study was conducted to evaluate the predatory potential of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) against aphids in tomato crop during the year 2008-2009 at entomological field area of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The standard agronomic practices were carried out during

the entire crop period in pesticide free conditions. Predator and prey relationship was studied on caged plants under natural field conditions. Second instar larvae of predator were released on caged plants in 1, 2, 3, 4, 5, 10, 15 and 20 numbers; whereas, the average aphid population was counted as 15/leaf/plant. After 6 days, aphid population was observed and recorded as 13, 10, 8, 8, 5, 3, 3 and 2 per leaf accordingly. Results conclude that aphid population can be suppressed below ETL (6 per leaf) by releasing 5 larvae of predator per plant.

**LIFE CYCLE AND HOST PREFERENCE OF COTTON MEALY BUG,
PHENACOCCLUS SOLENOPSIS T. (PSEUDOCOCCIDAE: HOMOPTERA)**

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Cotton (*Gossypium hirsutum* L.) is a major cash crop of Pakistan and contributes about 1.6 % to our GDP and 55% to our foreign exchange earnings. The life cycle and host preference studies of Cotton Mealy bug, *Phenacoccus solenopsis* T. were carried out at Agriculture Farm of University College of Agriculture, Bahauddin Zakariya University, Multan. Eight different host plants were selected including two crops (Cotton and Sunflower), two ornamentals (China rose and Flamingo Lily), two vegetables (Okra and Squash) and two weed species (Itsit and Lantana). Five replications were made for each host plant and in each replication, ten newly hatched nymphs or crawlers were put in a single petridish. Their food (small fresh twig bearing three to four fresh leaves) was changed regularly. The laboratory temperature was maintained at 25 ± 3 °C and relative humidity at 60 ± 5 %. The average life cycle of female and male cotton mealy bug on various host plants was in the range 30 to 34 and 13 to 17 days respectively. The shortest developmental period of female cotton mealy bug was 30 days on Okra and that of male was 13 days on China rose which showed that these are the most favourable hosts of cotton mealy bug. The longest developmental period of female cotton mealy bug was 34 days on Lantana and that of male was 17 days on Sunflower which suggested the unsuitability of these hosts. The highest survival of cotton mealy bug was found on Itsit while the lowest on the two host plants *i.e.* Flamingo lily and Lantana. The survival results showed that Itsit and China rose are the most preferable hosts of cotton mealy bug while Flamingo lily and Lantana are the least preferable.

EVALUATION OF GERMINATION LOSSES CAUSED BY MITES IN SEEDS OF MAIZE AND MUNG FROM FARMER'S HOLDINGS IN TEHSIL TOBA TEK SINGH

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The mites are important pests of stored grains and other stored commodities. They are responsible of both qualitative and quantitative losses in stored grains. These mites feed on embryo thus resulting in germination loss in the grains along with deterioration in quality of seed as well as flour prepared from the infested grains. The present studies were conducted to determine the impact of mite population on germination in seeds of Maize and Mung from farmers holdings of Tehsil Toba Tek Singh. The samples were collected from Tehsil Toba Tek Singh viz., Toba Tek Singh, Janiwala, Dabawala, Jalalpur, Dulum, Rajana, Bairianwala, Pairra and Chatiana. Mite population, germination and infestation were recorded initially and after three months of storage. The results revealed highly significant differences between pest mite populations at different places. In maize maximum and minimum initial and final population of harmful mites were recorded from Rajana which was 3.33 and 6.66 respectively. In mung, maximum initial and final population of harmful mites was 2.33 and 5.33 respectively, from Rajana. Significant variations were recorded in initial and final germination percentage of the both commodities which ranged 86-91, 74.75-81 respectively, in maize and 85.87-92.12, 75.87-83.37 respectively in mung. Similar trend was recorded in infestation of both commodities. Negative correlation was observed between harmful mite population and final germination percentage of maize and mung with correlation coefficients of -0.07 and -0.507 respectively.

BIOLOGICAL ACTIVITY OF FLUFENOXURON, A CHITIN SYNTHESIS INHIBITOR, ON VARIOUS STAGES OF RED FLOUR BEETLE DUE TO LARVAL FEEDING ON FLUFENOXURON TREATED-DIET

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Red flour beetle is an economically important pest of stored grain commodities through out the world. Flufenoxuron, a chitin synthesis inhibitor, was tested against two strains of *T. castaneum* at concentrations of 0.006, 0.003, and 0.0015 ppm by exposing 15-day old larvae of *T. castaneum* to flufenoxuron treated wheat flour under laboratory

conditions. Flufenoxuron caused significant effects on larval mortality, larval weight, larval period, pupation, adult emergence and finally on next progeny at all the concentrations against both the strains. Flufenoxuron caused 17.86% and 16.07% mortality of larvae at 0.006 ppm after 5 days of treatment for Multan and Faisalabad strains respectively. Similarly at 0.006 ppm larval weight was reduced by 8.76% and 8.88% of Multan and Faisalabad strains respectively. Larval period was prolonged significantly at all the concentration for both the strains. Pupation was severely affected, resulting in inhibition of 75.20% and 70.83% (at 0.006 ppm) for Multan and Faisalabad strains respectively. Emergence of adults was inhibited by 39.11% and 47.67% at 0.006 ppm for Multan and Faisalabad strains respectively. Growth inhibitory effects were continued to the next generation due to the transovarial activity of flufenoxuron, causing 94.19 and 97.11 percent reduction (at 0.006 ppm) in F1 adult population of Multan and Faisalabad strains respectively. Present results show the potential usefulness of flufenoxuron in storage houses and godowns for the management of red flour beetle and other stored grain pests.

**RESPONSE OF RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*
(TENEBRIONIDAE: COLEOPTERA), TOWARDS ESSENTIAL OILS OF TWO
MEDICINAL PLANTS AND SPINOSAD**

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Present studies were carried out to evaluate the insecticidal activity extracts of *Azadirachta indica* and *Nerium olender* along with a new chemistry insecticide, spinosad (tracer 240 SC) against *Tribolium castaneum* (Herbst). Adults of *Tribolium castaneum* (Herbst) were collected with the help of sieves from various godowns of Punjab Food Department located in Faisalabad. Collected insects were reared on wheat flour glass jars to get homogenous population at $30\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ R.H. Plants extracts were obtain using Soxhlet apparatus in acetone solvent. Five different concentrations viz. 0.5, 1.0, 1.5, 2.0 and 2.5% of each plant extract and spinosad were prepared in acetone. These test solution were applied on filter paper. After application, the filter papers were air dried and placed in the petri dishes. Twenty adults of *Tribolium castaneum* were released on the filter paper, after that petridishes closed tightly. All treatments were replicated three times along with one untreated control Data for percent mortality was recorded after 24, 48, 72, 96 and 120 hours respectively. At the end of each exposure period mortality was assessed. The collected data were analyzed statistically by analysis of variance and Duncan's Multiple Range Test. Results shows that mortality of *T. castaneum* was increased with the increase in concentration and exposure time. In case of *A. indica* and *N. olender*, percent mortality increased with the increase in concentration and exposure

time. Maximum mortality (32.5%) was observed after 120 hours exposure of insect to *A. indica* and 27.77% in case of *N. olender*. 33.33% and 26.66% mortality was observed at 2.5% concentration of *A. indica* and *N. olender* respectively.

**EFFICACY OF NEW CHEMISTRY INSECTICIDES AND PLANT EXTRACTS
AGAINST *TROGODERMA GRANARIUM* (EVERTS) (COLEOPTERA:
DERMESTIDAE)**

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The efficacy of plant products (Neem and Kalwanji) and new chemistry insecticides (Emamectin Benzoate and spinosad) were evaluated under the laboratory condition following completely randomized design. The experiment was carried out to check direct mortality and population build up. Patri dishes were used as exposure chambers. Concentrations of all the chemicals and plant extracts were prepared in acetone. 2, 3 and 4% concentrations of Neem and Kalwanji while 5, 10, and 15 ppm of Emamectin Benzoate and spinosad were used. Mortality data was recorded after 3, 5 and 7 days while post treatment build up of population was recorded after 35 and 70 days. Results showed that Emamectin benzoate showed more mortality than Spinosad and population build up was less in Emamectin benzoate as compared to spinosad. Neem is more toxic than Kalwanji. Neem showed more mortality and less population build up as compared to Kalwanji

**INSECTICIDAL ACTIVITY OF *ALLIUM SATIVUM* (GARLIC), *ZINGIBER OFFICINALE* (GINGER) AND *NIGELLA SATIVA* (KALWANJI) AGAINST
TROGODERMA GRANARIUM EVERTS (COLEOPTERA: DERMESTIDAE)**

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Studies were carried out to evaluate the insecticidal activity of *Allium sativum*, *Zingiber officinale* and *Nigella sativa* extracts, each in concentration of 2, 4, and 6 percent against *Trogoderma granarium* Everts. The studies were carried out under the laboratory condition at 30 ± 32 °C and 70 ± 75 % R.H., following Complete Randomized Design. The filter papers were soaked in solutions of extracts and forty grubs of *Trogoderma granarium* were released in each petri dish. In the highest concentration of 6

% *Zingiber officinale* (24.60 % mean mortality) was found more toxic than *Allium sativum* (15.97 %) and *Nigella sativa* (8.24 %) at 96 hours exposure time. Regarding latent effect of the test plant materials, *Nigella sativa* (65.82 %) and *Zingiber officinale* (54.14 %), both being statistically at par with each other, gave significantly higher reduction in F1 generation than *Allium sativum* (32.19 %).

**STUDY OF PREDATION RATE IN SELESTED COCCINELLID
(COLEOPTERA) PREDATORS ON SOME MAJOR APHIDID AND
CICADELLID (HEMIPTERAN) PESTS**

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Predation rate of adult and larvae of *Coccinella septumpunctata*, *Cheilomenes sexmaculata*, and *Hippodamia variegata* on aphids *Macrosiphum miscanthi*, *Schizaphis graminum*, *Diuraphis noxia* and jassid *Empoasca kerri*, the major pests in croplands of Punjab was studied in laboratory. The experiments were carried out at $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$, at relative humidity of 60 ± 5 and 11:13 (light: darkness) hours photoperiod. Each predator was fed on single prey species at a time. Different level of prey densities 30, 40 and 50 were used. The average predation rate ranged from 10.6 to 35 aphids and 6.4 to 25.2 jassid per day. Predation rate of each predator species increased with the increase in prey density of each species. Overall larval feeding rate was 2.0 fold greater than those of adults. Adult *Cheilomenes sexmaculata* and *Hippodamia variegata* had greater predation rate as compared to *Coccinella septumpunctata* whereas the larval feeding rate was almost same within each prey density.

**STUDY OF REPELLENCY AND TOXICITY OF MIRAGE (IMIDACLOPRID)
AND TORQUE (CHLORPYRIFOS) AGAINST *MICROCEROTERMES*
CHAMPIONI (SYNDER)**

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Different concentrations of Mirage and Torque were applied to soil to test its toxicity and efficacy against *Microcerotermes championi* (synder). Mortality during 8 hours exposure for torque at different concentrations 1.875, 3.75, 7.5, 15, 30, 60, 120, 240, and 480ppm were 50%, 63.3%, 63.3%, 66.6%, 66.6%, 70%, 76.6% and 83.3% and for Mirage at different concentrations 25, 50, 100 and 200ppm mortality was 13.33%, 20%, 26.6% and 33.3% respectively. Toxicity of Torque (chlorpyrifos) and Mirage

(imidacloprid) was compared. Torque was found to be more toxic than Mirage. The LC_{50} value for *M. championi* exposed to soil treated with Torque and Mirage were 1.862ppm and 741.3ppm respectively.

STUDY ON THE EFFECT OF PREVENT (FIPRONIL) ON MICROCEROTERMES CHAMPIONI (SYNDER)

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Present study involves the effect of prevent (fipronil) on *Microcerotermes championi* (Synder), a subterranean termite, widely distributed in Pakistan. The toxicity of Prevent (fipronil) applied to soil was recorded against *M. championi* according to Smith 1979. Mortality during eight hour exposure for prevent at 1.95, 3.90, 7.81, 15.62, 31.25, 62.5, 125 and 250ppm was 10%, 23.33%, 33.33%, 46.66%, 53.33%, 56.66%, 76.66% and 86.66% respectively. The LC_{50} for *M. championi* was 28.18 ppm. In repellency test soil treated with prevent was repellent at 1.95, 3.90, 7.81, 15.62, 31.25, 62.5, 125 and 250ppm concentration.

POST SPRAYED EFFECTS OF INSECTICIDES ON AGROBIONT SPIDERS OF RICE FIELD

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Spiders are among the most abundant predators of pests in various agrobiocenoses. Field application of insecticides often led to a reduced density of spiders for several weeks. In this study we evaluate residual activity of selected insecticides on the mortality and behavior of two spider species (*Lycosa terrestris* and *Pardosa birmanica*). Two insecticides, thiodan (cyclodiene) and lambda cyhalothrin (pyrethroid) were used at the field application rate. Mortality was recorded after exposure to spiders to fresh (2h), 5, 10 and 20 days old residues. For each residue mortality was evaluated for 8, 24 and 48 hours. Results showed that effect varies with residue age, species of spider and insecticide. In both species of spiders, mortality decline with the age of the residues. However, *P. birmanica* was more susceptible and repellent to insecticides than *L. terrestris*. The data of locomotion also showed that after exposure to fresh residues of insecticides the movement of both spider species increase as compared to control. However, in specimens exposed to old residues of insecticides, movement pattern was similar to control. In conclusion, aged insecticides residues possess a high activity and can cause decrease in abundance and disturbance in behavior of spiders.

**EFFECT OF SOME INSECTICIDES ON DETOXIFYING ENZYMES IN
OXYOPES JAVANUS (ARANEAE: OXYOPIDAE)**

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The present study was conducted to check the effectiveness of detoxifying abilities of lynx spider (*Oxyopes javanus*), after exposure to different concentrations of three insecticides, Chlorpyrifos (organophosphate), Lambda cyhalothrin (synthetic pyrethroids), Imidacloprid (neonicotinoids), in response to time and dose. For this purpose the activities of three enzymes, acetylcholinesterase, carboxylesterase and glutathione S-transferase were assessed in test organism against four concentrations ($1/5^{\text{th}}$, $1/10^{\text{th}}$, $1/15^{\text{th}}$ and $1/20^{\text{th}}$ of the field rate) of Chlorpyrifos, Lambda cyhalothrin, Imidacloprid at 24, 48, and 72 hours of exposure time. Probit analysis was carried out on five days mortality observations for each insecticide. Results showed that Lambda cyhalothrin and Imidacloprid are more toxic as compared to Chlorpyrifos. In the other experiment activities of enzyme was assessed. Results showed that levels of acetylcholinesterase, carboxylesterase and glutathione S-transferase strongly change with dose and time in response to each insecticide. All the results emphasize that all insecticides are highly toxic for spiders and highly disrupt the natural biological control system related to spiders. So there is a need to reduce or minimize such compounds from the field by applying other tactics.

**COMBINED EFFECTS OF TWO INSECT GROWTH REGULATORS AS
DEVELOPMENTAL STAGES INHIBITORS OF THE TERMITE
MICROCEROTERMES DIVERSUS (SILVESTRI) (ISOPTERA:TERMITIDAE)**

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Chemical treatment with persistent organochlorine insecticides is the most frequently used strategy to control subterranean termites in developing countries of the world. Cellulose baits impregnated with Throughout the world, Insect Growth Regulators (IGRs) are used as a part of integrated termite management practices. *Microcerotermes diversus* (Silvestri) is the most economically destructive termite in structures in Iran. The combined effects of two IGR active ingredients on mortality, incomplete ecdysis and intercaste production in larvae, workers and nymphs of *M. diversus* were assessed under feeding trials over a range of concentrations (10-5000 ppm). All concentrations of flufenoxuron greater than 100 ppm elicited 100% mortality of the workers. In all concentrations of this compound larvae and nymphs died due to direct toxicity or during

metamorphosis (molting failure). All concentrations of fenoxycarb produced nymph – presoldier intercastes with the exception of the lowest concentrations. These assays determined concentrations of fenoxycarb and flufenoxuron to be used in bait formulation. These adverse effects including differentiation of nymphs into intercastes may cause disruption of the caste balance in *M. diversus*, leading to the collapse of the colony.

PREDATORY EFFICACY OF LADYBIRD BEETLE, *COCCINELLA SEPTEMPUNCTATA* (L.) ON CABBAGE APHID, *BREVICORYNE BRASSICAE* L. AND TURNIP APHID, *LIPAPHIS ERYSIMI* (KALT.) UNDER LABORATORY CONDITIONS

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Predatory efficacy of larvae and adults of ladybird beetle, *Coccinella septempunctata* (L.) was investigated under laboratory conditions at room temperature during months of February-March at Dera Ghazi Khan during 2006 on cabbage aphid, *Brevicoryne brassicae* L. and turnip aphid, *Lipaphis erysimi* (Kalt.). Twenty five nymphs of both the species were collected from field grown crop of *Brassica napus* L. and put in a petri dish of 5 cm diameter. Experiment was replicated three times. In each replication nymphs were offered in 10 petri dishes to a larva or an adult of *C. septempunctata*. Mean numbers of nymphs consumed daily were counted. *C. septempunctata* consumed 4.93, 6.96, 11.21, 11.70 & 15.60 and 5.5, 7.26, 10.85, 12.9 & 15.78 nymphs of *B. brassicae* and *L. erysimi* in 1st instar, 2nd instar, 3rd instar, 4th instar and adult stage, respectively.

EFFICACY OF PLANT EXTRACTS ON POPULATION DYNAMICS OF *AMRASCA BIGUTULLA BIGUTULLA* I AND *THRIPS TABACI* L ON COTTON (*GOSSYPIUM HIRSUTUM*)

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Jassid and thrips have become major pests in subcontinent due to invasion of Bt cotton. Insecticidal resistance by these pests is another threat. Keeping in view these facts, five plant derivatives viz., Lemon oil, Bittergourd extract, bakain leaf extract, Neem oil and Neem leaf extract each @ 5% concentration were tested for their repellency and

phagodeterrent effects against jassid and thrips with three applications upon emergence of pest population. The variety CIM-496 was sown during 2008 at experimental area of the department of Agriculture, Entomology, University of Agriculture, Faisalabad. Six treatments including control were maintained following Randomized Complete Block Design with three replications. The plant derivatives were applied three times at the interval of 20 days. Data was recorded 24, 48, 72, and 7 days intervals after application of each botanical. Results revealed that lemon oil proved to be the most effective plant derivatives against jassid followed by neem oil, bakaine, bitter gourd and neem leaf extract in all three applications. In case of thrips all plant derivatives controlled the thrips population significantly but the response was different in different post treatment intervals. Furthermore, bakain leaf extract, neem oil and neem leaf extract showed comparatively higher mortality of the pest as compared to other plant derivatives.

EFFECT OF VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES ON THE BIOLOGY OF COTTON MEALY BUG *PHENACOCCLUS SOLENOPSIS* TINSLEY (STERNORRHYNCHA: COCCOIDEA: PSEUDOCOCCIDAE) IN PAKISTAN

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Cotton mealybug (*Phenacoccus solenopsis* Tinsley) is a divesting pest attacking more than 150 crops in the subcontinent, emerged as a key pest during 2005 in Pakistan. Studies were conducted on biology of cotton mealy bug in laboratory at Entomological Research Institute, Faisalabad during 2007-2008. Experiment was laid out in Completely Randomized Design (CRD) consisting of seven treatments and 14 replications. Each replication consisted of a plastic vial containing moistened sand, a fresh China rose stick embedded in the sand covered with plastic lid containing sieve of 90-mesh sizes. Adult female was also released in petri-dishes (9-cm dia) containing fresh leaves of cotton crop. Egg laying duration was checked after 8 h intervals to record the hatching time. Biology of pest was studied at seven different temperatures and relative humidities viz., 20°C at 70 ± 5% R.H, 25°C; 65±5% RH, 27°C; 63±5%, 30°C; 60 ±5% R.H, 32°C; 58± 5% R.H , 35 C ; 55 ±5% R.H and 40°C; 40±5%. Incubation period significantly decreased with increase in temperature from 32.0 hours at 20°C to 0.57 hours at 40°C. life period of first, second and third instar was decreased from 8.31 days to 3.0 days, 8.61 to 2.71 days and 7.4 to 2.4 days at 20°C at 70 ± 5% R.H and 40°C; 40±5%. Pupae duration decreased from 8.5 days at 20°C to 4.14 days at 35°C, while no male emerged from pupae at 40°C. Adult male life decreased from 1.9 days to 0.78 days at 35°C. Total male life decreased from 25.0 days at 20°C to 11.0 days at 35°C, while no male emerged at 40C Adult female life decreased from 90.5 days at 20°C to 12.7 days at 40°C. There was significant effect of temperature on fecundity of adult female. Total adult life (both male and female) was

prolonged at lower temperatures and shortened at higher temperatures. Optimum temperature for growth and development of the pest was 35°C; 55 ±5% R.H

MANAGEMENT OF PEACH FLAT- HEADED BORER BY USING IPM COMPONENTS IN PLUM

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The climate of N.W.F.P. is very congenial for production of different types of fruits especially stone fruits such as peach, plum & apricot. The total area under these fruit orchards in NWFP was 11.9 thousand hectares, which produced 111.8 thousand tones of fruit (Anonymous, 2007-08). Various insect pests attack these fruit trees but peach flat-headed borer is the serious one causing gummosis, a major problem resulted in declining fruit growing area in NWFP. Our farmers solely relied on the frequent use higher doses of agric-pesticides and the pest control situation are quite alarming. Current project is aimed to develop IPM model based on chemicals/ cultural practices, determination of proper insecticides/fungicides & proper application timings based on weakest link of pest life cycle towards better management in stone fruit orchards. Effectiveness of different insecticides Le., Cyren 48EC, Perfekthion 40EC, Thiodan 35"EC, Tenakil 40EC, Foliar summer oil-& Triazofos in Bordeaux mixture @ 100 ml of each mixed with the same dose of fungicide (Copper oxychloride) + NaCI + Lime + water @ 100g + 28.5g+3 kg +10L were painted on plum tree trunks against peach flat headed borer in the month of January. Results showed that the minimum infestation was recorded in the Cyren Le., 0.02 gum points/m² followed by Tenakil (0.07), Perfekthion (0.08), Foliar summer oil (0.09), Thiodan (0.10) & control (0.11) respectively. Cyren is very effective in Bordeaux mixture as compared to other insecticides. Similarly, detection of adult emergence timings of Pest in plum orchard was carried out. Adult trapping was done by covering 10 branches of trees with plastic strips. Data were taken on weekly basis throughout the year. Results showed that maximum adult emergence occurred in the 3rd week of April Le., 2.30 adults/plastic strip followed by 2nd week of June (1.10) and no adult emergence activity was recorded in other days. After adult emergence, different synthetic insecticides with fungicide against Peach flat-headed borer were tested. The efficacy of Cyren 48EC, Perfekthion 40EC, Thiodan 35EC, Tenakil 40EC, Foliar summer oil & Triazofos @ 50 ml of each mixed with the same dose of fungicide (Copper oxychloride) @ 50g/10L of water were sprayed on plum tree trunks. The data showed that the minimum infestation was recorded in the Cyren i.e., 0.03 gum points/m² followed by Tenakil (0.06), Thiodan (0.06); Perfekthion (0.07), Triazofos (0.09), Decis-D (0.18) and control (0.28) respectively. Cyren efficacy is very high as compared to other insecticides during field spray.

RESPONSE OF *TRIBOLIUM CASTANEUM* TOWARDS DIFFERENT CONCENTRATIONS OF PLANT EXTRACT AND SPINOSAD

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The persistence and insecticidal activity of a commercial biological insecticide Spinosad (based on fermentation product of Actinomycetales bacterium *Saccharopolyspora spinosa* (Actinomycetales: Actinomycetaceae) and two plant extracts viz., Neem (*Azadirachta indica*) and Kanair (*Nerium oleander*) were evaluated against *Tribolium castaneum* (Hbst) on stored wheat grains. Five concentrations viz: 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% of each insecticide were used at different exposure times. i.e. 24 hours, 48 hours, 72 hours, and 120 hours. Filter paper dip method was used. Results revealed that Spinosad (Tracer 240 SC) was the best against target pest, with maximum mortality i.e. 55% @ 2.5% dose in 120 hours exposure time and minimum 16.664% with 0.5% concentration at 24 hours exposure time. Neem showed 45% mortality at 120 hours exposure time with 2.5% concentration and 16.667% at 0.5% dose at 24 hours exposure time followed by Kanair with 38 % mortality at maximum application rate and exposure time. i.e. 2.5% and 120 hours and minimum 15% at minimum application rate. i.e. 0.5% at 24 hours exposure time. Results revealed that biological and botanical insecticides are better ways to manage Red flour beetle infestation in stored wheat grains.

MONITORING OF RESISTANCE IN *HELICOVERPA ARMIGERA* AGAINST INSECTICIDES WITH NOVEL MODES OF ACTION

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Insecticides resistance in 2nd instar larvae of *Helicoverpa armigera* was measured by leaf dip method. Larvae of *Helicoverpa armigera* collected from different locations of Punjab, i.e. Multan, Muzzafargarh, Vehari and Bahawalpur. Insecticides, i.e. lufenuron, abamectin, emamectin benzoate, spinosad and methoxyfenozide were used at field recommended doses. Insecticide solutions were prepared in distilled water. Population of *H. armigera* collected from Multan was noticed highly susceptible against all the insecticides followed by the populations from Vehari, Muzzafargarh and Bahawalpur. *H. armigera* collected from Bahawalpur showed high levels of resistance, whereas populations of all four locations demonstrated resistance against abamectin, spinosad,

lufenuron and methoxyfenozide at lower to moderate levels. Populations from Bahawalpur and Vehari showed high levels of resistance against emamectin benzoate as compared to other insecticides, while all populations showed lower levels of resistance against spinosad. It was suggested that insecticides with novel mode of action may be used for the management of *H. armigera*.

CURRENT SCENARIO OF INSECTICIDE RESISTANCE IN *HELICOVERPA ARMIGERA* (HÜBNER) AGAINST CONVENTIONAL INSECTICIDES

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The study was conducted to check the current scenario of insecticide resistance in *Helicoverpa armigera* against conventional insecticides (endosulfan, profenofos, carbosulfan, deltamethrin) by two bioassay techniques *i.e.* residual method (leaf dip) and topical method. *H. armigera* from Southern Punjab prevailed low to moderate resistance against the conventional insecticides. Data suggests that multiple resistance also prevails in the field populations of *H. armigera*. The toxicity of the conventional insecticides in residual method was non-significantly different from that of topical method. Resistance ratios in topical method were found higher as compared to the residual method, which may be attributed to the delayed cuticular penetration and enhanced metabolism of the insecticides. It can be concluded that the rotational use of conventional insecticides along with the new chemistry insecticides may be an effective tool in the insecticide resistance management programs.

BASELINE SUSCEPTIBILITY AND STABILITY OF INSECTICIDE RESISTANCE IN *SPODOPTERA LITURA* (F.)

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A population of *Spodoptera litura* collected from dunyapur was reared for 11 generations under laboratory conditions without any insecticide exposure. The LC₅₀ data was recorded through diet incorporation method at 1st, 4th, 5th, 7th, 8th, 9th, 10th and 11th generation against four insecticides (emamectin benzoate, spinosad, imidacloprid and profenofos). Emamectin benzoate (3.85 ppm) was found to be most toxic on the basis of LC₅₀ values followed by spinosad (7.77 ppm), profenofos (686.5 ppm) and imidacloprid (258.75 ppm) at F1. The decrease in the LC₅₀ values after 11 generations as compared to

the field population of *S. litura* were 11.40-, 9.83-, 9.3- and 13.82-folds against emamectin benzoate, spinosad, imidacloprid and profenofos, respectively. There was a significant rate of decrease in insecticide resistance against all the test insecticides. The estimated 10-folds decrease in resistance was after 11.36, 11.11, 10.41 and 9.61 generations for imidacloprid, spinosad, emamectin benzoate and profenofos, respectively. This baseline susceptibility data could be very helpful in future monitoring of insecticide resistance in *S. litura*.

**MANAGEMENT OF MANGO MEALY BUG (*DROSICHA MANGIFERAE*)
CRAWLERS BY PESTICIDES**

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Mango mealy bug (*D. mangiferae* G. Homoptera: Margarodidae) is a serious pest of mango orchards. Insecticides viz., Pirate 360Sc, Cascade 10 WDC, Confidor 20SL, Acetamiprid 20SP, Buprofezin 25% W/W, Karatae 2.5 EC, Decis 10EC and Sevin 85SP were applied on 1st and 2nd instar crawlers of *D. mangiferae*. Insecticide concentration were prepared in distilled water and applied by leaf dip method using mango leaf discs 3cm in diameter. The data revealed that out of eight insecticides tested against 1st instar crawlers, Buprofezin, Karatae and Acetameprid showed 100% mortality after 7 days of treatment. Buprofezin, Decis and Sevin caused 100% mortality to 2nd instar, after 5 and 7 days respectively. Pirate showed minimum percentage mortality to 1st and 2nd instar crawlers. The insecticides showing maximum effect against this insect may be used for the management of mealy bugs, keeping in view their compatibility with other control measures and their hazards to the environment.

**IMPACT OF LIGHT TRAPS ON POPULATION DENSITY OF GRAM POD
BORER (*HELICOVERPA ARMIGERA* (HUB.) LEPIDOPTERA; NOCTUIDAE)
AND EMERGENCE RATE OF ITS LARVAL PARASITOID (*CAMPOLITUS
CHLORIDAE*) IN ROD KOHL AREA OF DERA ISMAIL KHAN**

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Three treatments, T1 (one light trap per hectare), T2 (two light trap per hectare) and T3 (control) were tested against gram pod borer (*Helicoverpa armigera* Hub.) and emergence rate of its larval parasitoids under field conditions at Dera Ismail Khan. The

data were recorded on the basis of larval population per plant, pod damage and yield. Percent parasitism and relationship of light traps on the population of parasitoids were also studied. Two light traps per hectare (T2) captured average (45.26) *H armigera* moths with lowest larval population (0.25/plant), lesser pod damage (4.02%) and higher yield (2120 kg/ha), while T1 (one light trap per hectare) resulted in average maximum 49.0 captures of *H armigera* moths with average larval population of 0.306/plant, 5.46% pod damage and 1980 kg/ha average yield. Maximum larval population density of 0.379 larva/plant, 10.40% pod damage and significantly lowers 1834 kg/ha was recorded on T3 (control). On the average, T1 captured 5.82 larval parasitoids while T2 resulted in 5.19 larval parasitoids. Maximum percent emergence of larval parasitoids (9.047) of *H armigera* was recorded in reared larvae on T3 (control) followed by T1 with 7.016% parasitized larvae while T2 gave minimum (7.0%) larval parasitism. Highest average percent pupation and larval mortality were recorded in T1 (84.27; 9.58) followed by T2 (82.97; 10.11) and minimum in T3 (80.09; 10.86) respectively.

**PRELIMINARY STUDIES ON PERFORMANCE OF NUCLEAR
POLYHEDROSIS VIRUS (NPV) AGAINST CHICKPEA POD BORER ON
CHICKPEA CROP**

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Helicoverpa armigera (Hb.) is a major Lepidopteran economic pest of chickpea, tomato, cotton and tobacco crops of Pakistan. Chickpea crop is severely damaged by pod borer, *Helicoverpa armigera*. Pest damage at the podding stage is extensive, ranging from 20-96%. The caterpillars damage the crop during vegetative and podding stages. Farmers increasingly rely on synthetic insecticides to manage this pest in different crops leading to develop insect resistance to pesticides. Nuclear Polyhedrosis Virus (NPV) is a naturally occurring viral disease of pod borer larvae that causes heavy mortality but no deleterious effect on non-target insects. NPV is safe and environmentally friendly. The product is produced from the culture of infected pod borer larvae under controlled conditions. A field trial was carried out to evaluate the efficacy of HNPV s for management of pod borer on chickpea crop. Formulation of nueleo polyhedrovirus @ of 200 ml/ha having a dose of 0.2% concentration was applied at the onset of pod borer larval attack. Data were recorded on damaged and undamaged pods from randomly selected ten plants in each plot including control (untreated plot). The results indicated that pre-treatment counts were 6.1larvae/plant while post-treatment larval populations recorded were 1.7 larvae/ plant. Pods damage in treated plot was 10.74% while in control (untreated) it was found 51.45%. Grain yield was maximum (1102 kg/ha) than control (593 kg/ha). Based on the results obtained, spraying of HNPV can effectively reduce the number of larvae associated with pods damage in chickpea crop.

PHENOLOGICAL RESPONSE OF COTTON MEALYBUG *PHENACOCCLUS SOLENOPSIS* TINSLEY (STERNORRHYNCHA: PSEUDOCOCCIDAE) TO THREE PROMINENT HOST PLANTS

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The phonological studies of cotton mealybug *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) were carried out on three host plants of economic importance (cotton *Gossypium hirsutum*, china rose *Hibiscus rosa-sinensis* and okra *Abelmoschus esculentus*) under controlled environmental conditions (400 C, 65%RH and 10:14 light:dark regime). The development of all female nymphal instars was more rapid on cotton followed by okra and china rose, whereas the development of male nymphal instars was fastest on china rose followed by okra and cotton. Female instars lasted for longer than those of male on all tested hosts. Duration of 1st, 2nd and 3rd male nymphal instars on cotton was 4.17, 4.00 and 5.95 days and 3.93, 4.20 and 7.00 days for female respectively. Total longevity of mated and unmated females was 38.17 and 51.20 days on cotton, respectively followed by on china rose (36.80 and 46.90 days) and on okra (34.67 and 41.29 days). Total longevity of male was 21.64 days on okra followed by 21.33 and 20.20 days on cotton and china rose, respectively. The pre oviposition period was 9.50 days on cotton, 8.40 days on china rose and 8.00 days on okra. Maximum number of eggs 264.83 were laid on cotton followed by okra 152.00 and china rose 137.60. Similarly maximum total number of egg layings 5.88 was observed on cotton followed by 5.80 on china rose and 4.66 on okra. Egg laying period was 9.80 days on china rose followed by on cotton 8.00 days and on okra 7.33 days.

BIOETHOLOGICAL STUDIES OF COTTON MEALYBUG *PHENACOCCLUS SOLENOPSIS* TINSLEY (STERNORRHYNCHA: PSEUDOCOCCIDAE) AT THREE TEMPERATURE REGIMES

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In Pakistan the control of cotton mealybug *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) got an impetus since it has gained the status of

damaging pest of cotton in 2005, when first recorded. Knowledge of biology of *P. solenopsis* is important for designing a sustainable integrated management program. Development and reproduction of *P. solenopsis*, on cotton (*Gossypium hirsutum* L.), were investigated at three constant temperatures *i.e.* 20, 30 and 40°C. The duration of all the developmental stages of females was shortest at 40° C followed by 30° C and 20°C respectively, whereas, males had shortest developmental stages at 30° C followed by 40 and 20°C. Adult males lived for 5.53, 3.00 and 2.13 days at 20' 30 and 40° C respectively. Longest Pre-oviposition period was observed at 20° C (15.60 days) followed by 40° C (9.41 days) and 30°C (5.53 days). The tested temperatures seem not affecting the total reproductive days of mated females. Females laid the highest total eggs (352.9); number of egg laying (8.80) and number of eggs per laying (39.07) at 30°C. Total longevity of mated and unmated females was (56.27 and 68.57 days respectively) highest at 20°C.

THE RESPONSE OF THE DIFFERENT RICE VARIETIES AGAINST THE WHITEBACKED PLANTHOPPER, *SOGATELLA FURCIFERA* (HORVATH) (HEMINOPTERA: DELPHACIDAE) UNDER FIELD AND GREEN HOUSE CONDITIONS

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Twenty two rice varieties, 6 medium maturing, 7 early maturing coarse rice and 9 fine rice varieties were tasted against the Whitebacked Planthopper, *Sogatella furcifera* (Horvath) under field and green house conditions. The experiments were conducted at Rice Research Institute, Dokri during the year 2008. The experimental results show that Medium Maturing rice varieties are more susceptible for WBPH than early maturing and Fine rice varieties. Fine rice varieties are more resistant for WBPH than the Early and Medium Maturing coarse rice varieties.

EFFECACY OF DIFFERENT SYNTHETIC AND NATURAL PRODUCTS AGAINST THE OUTBREAK OF MEALY BUG (*MACONELLICOCCLUS HIRSUTUS*) IN COTTON

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For devising environmentally safe chemical based management strategy against the severe outbreak of mealy bug in cotton, efficacy of different natural products; tobacco (nicotine), neem oil, neem seed kernal extract, garlic extract and surfactant were

compared to synthetic chemicals; commercial systemic insecticide (Curacron), Isopropyl alcohol, Ethanol and alcohol in combination with surfactant at different concentrations. Based on the results tobacco solution and neem oil @ 2% & 1 % respectively, among the natural products, and insecticidal spray of Curacron at recommended dose and alcohol + surfactant. @ 10 % were found useful in suppressing the pest population ephemerally. However, the population was found to be restored within 7-10 days period after any treatment. The uses of insecticide curacron and natural product tobacco are found at par in term of mortality of the mealy bug. However, the resurgence of the mealy bug was found in similar fashion in all the synthetic and natural products. These results highlighted the need for further evaluation of different natural and synthetic chemicals in the control of this notorious pest.

**EFFECT OF *AZADIRACHTA INDICA* (NEEM) PLANT EXTRACT AND
CAFFEINE AS A BOTANICAL PESTICIDE ON THE TERRESTRIAL SNAILS**

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The tree of *Azadirachta indica* is native to parts of South Asia, where it has been used for many things. *Azadirachta indica* is considered to be of the most ecological importance. As an alternative for toxic pest repellents and for safe pests control methods the neem tree is more suitable for pest management system. *Azadirachta indica* has been tested for the effect against larvae and adult stage of the snail. Other natural product, considered to be good as compared to the chemical pesticides, such as liquid metaldehyde is caffeine. The extracts of *Azadirachta indica* and caffeine were used in the study to investigate the feeding preference of snails (*Lymnaea acuminata*) which shows the feeding of snails at 4 percent and 2 percent (choice test choice and no choice tests). Data was analyzed by one way ANOVA and results of feeding of snails for choice ($P=0.00815$) and no-choice ($P=0.025$) at 4 percent of neem extract were significant. In the same manner, the result of feeding on snails for choice ($P=0.04$) and nochoice ($P=0.04$) at 2 percent of neem extract were also significant. It shows that at both percentages, Neem has reduced the feeding of snails. The result was analyzed by two factors ANOVA without replication and result of the comparison of caffeine and water on snails was significant [$p < 0.05$]. This value showed that caffeine was effecting on snails. And we had also used the water-based Neem solution of different percentages to check the effect of Neem on the mortality of snails. But even at 50 percent, no mortality was observed. However when compared with caffeine it was found that P value for effect of caffeine on snails mortality after 24 hours is 0.0002 and is less than 0.05 which means caffeine has molluscicidal effect on snails and mortality of snails increased with increasing concentration of water based caffeine solution. P value for effect of caffeine

on snails mortality after 24 hours is 0.0001. This value is less than 0.05 means results are significant and caffeine act as toxicant against snails.

STUDY ON PARTHENOGENESIS IN COTTON MEALY BUG (*PHENACOCCLUS SOLENOPSIS*)

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A study was carried out to monitor the parthenogenesis in Cotton mealy bug (*Phenacoccus solenopsis*) upon two hosts (Cotton and China rose). Newly hatched crawlers of mealy bug were individually reared twice in a season in plastic Petri dishes containing host under control condition $25\pm 2^{\circ}\text{C}$, 14:10 L:D and $65\pm 5\%$ RH at Bio-Control Laboratory of IPM Station (PARC) RZ. University, Multan during the year 2009. Half of the females were provided males for mating while other females remained without males. The females without males did not produced any offspring as compared to females which were provided with males produced 317 ± 9 and 415 ± 12 eggs per female on Cotton and China rose, respectively. There was no significant difference in the longevity of fertilized and unfertilized females. It can be inferred from the experiment that there is no parthenogenesis occurs in cotton mealy bug and experimentation is needed on male capturing techniques of Cotton mealy bug for its environment friendly management.

POTENTIAL OF CITRUS SEED EXTRACTS AGAINST *AEDES ALBOPICTUS* (SKUSE) (CULICIDAE: DIPTERA) LARVAE

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Temperature and more urbanization have led to issues like improper disposal of wastes, and breeding of pests. Every year huge amount of money is spent on the pesticides for the treatment of these waste yards and temporary water catchments. Still there are cases where organochlorinated hydrocarbons are widely being used. Plant extracts generally have essential oils with insecticidal activity. Such activities from Rutaceae particularly citrus have attracted greater concern due to their growth inhibiting effects against insect pests. Therefore the extracts from citrus varieties with insecticidal qualities were used against immatures of *Aedes albopictus* (Skuse). The results have revealed that rough lemon *Citrus jambhiri* and lemon *Citrus limon* had the lowest LC_{50} values (119.99 and 137.26 ppm respectively), after 24 hours of exposure, followed by red

blood *Citrus sinensis* (295.63 ppm), chakutra *Citrus grandis* (334.87 ppm), galgal *Citrus pseudolimon* (644.25 ppm), brazilian sour *Citrus aurantium* (905.96 ppm) and kinnow *Citrus reticulata* (1022.67 ppm). Narangi *Citrus mitis* had the highest LC₅₀ value (2069.117 ppm) after 24h of exposure followed by grape fruit *Citrus paradise* (1598.15 ppm) and musambi *Citrus sinensis* (1389.16 ppm), implying their least effectiveness against *Aedes albopictus* larvae. Percent mortality of *Aedes albopictus* larvae after 24h of exposure under lab conditions showed that lemon and rough lemon gave the highest percent mortality (88.9 and 95.6% respectively). Narangi (11.7%) showed the least percent mortality. After 48h, rough lemon and lemon gave almost 100% mortality (99.6 and 95.6% respectively). There was also a statistically significant difference among different citrus seed extracts in relation to time to kill. Rough lemon (LT₅₀, 2.51h) was the fastest killer followed by lemon (LT₅₀, 4.91h) and red blood (LT₅₀, 8.52h). Galgal showed 50% mortality after 26h; kinnow and musambi took almost two days (50.3 and 52.7h respectively) to give 50% mortality. Grape fruit took almost three days to cause 50% mortality. So it is clear that rough lemon, lemon and red bloods are the best varieties in terms of LC₅₀, % mortality and L T so while narangi is the least effective variety in terms of LC₅₀ and %mortality. Our studies, therefore, suggest that citrus seeds have essential oils which have greater potential in the management of *Aedes albopictus*.

EFFICACY AND PERSISTENCE OF BIOINSECTICIDES IN COMPARISON WITH CONVENTIONAL INSECTICIDES AGAINST WHITEFLY (*BEMISIA TABACI* GENN.) AND JASSID (*AMRASCA DEVASTANS* DIST.) ON OKRA CROP

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Recognizing the importance of organic farming and demand for safe food globally, present study was carried out to evaluate the effectiveness of bioinsecticides (biosal- neem formulation and spinosad) in comparison with conventional insecticides (imidacloprid, endosulfan and profenophos) against jassid and whitefly on Okra crop. The crop was sown in a randomized complete block design with three replicates each having six treatments including control. After pest infestation the crop was sprayed. Pre treatment insect count was recorded before 24 hours and post treatment count was made after 24, 72 and 168 hours of spray. All the three conventional insecticides were effective against jassid (efficacy: imidacloprid, endosulfan and profenophos = 91, 75, and 68% respectively) and whitefly (93, 82, and 74% respectively). Biosal showed moderate effectiveness (55 and 50% against white fly and jassid respectively), whereas spinosad was moderately effective against whitefly (46%) but less effective against jassid (29%). However conventional insecticides were persistent in the crop (half life: 1.95, 2.41 and

1.57 days for imidacloprid, endosulfan and profenophos respectively) while among bioinsecticides, spinosad was more persistent (half life: 1.25 days) than of biosal (half life: 0.27 days).

TRITROPHIC INTERACTIONS BETWEEN HOST PLANTS, APHID SPECIES AND LADY BEETLE, *MENOCHILUS SEXMACULATUS* (FAB)

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The adults of lady beetle, *Menochilus sexmaculatus*(Fab) were collected from mustered field. The culture was maintained on mustard aphid, *Lipaphis erysimi* (Kalt) under laboratory conditions. Eggs laid by female, *M sexmaculatus* were collected and kept in Petridishes lined with Whatman filter paper and a wet cotton wool swab was provided in Petri dishes to provide moisture. After hatching, neonate (10 hrs) grubs were introduced into Petridishes containing different host plants along with aphid species: mustard aphid, *Lipaphis erysimi* (Kalt); nerium aphid, *Aphis nerii* Boyr., Akk aphid, *A. nerii*, barley aphid, *Macrosiphum avenae* (Fitch) and tobacco aphid, *Myzus persicae* (Sulzer) . Aphid species along with fresh host plant leaves were provided as a food (billy) in Petridishes for feeding and old leaves along with remaining aphids were removed from the Petridishes. There were four replications for each treatment; each treatment was replicated ten times and the experiment was conducted for four generations. Incubation period of beetles varied from 2.00 to 4.33 days when fed on tobacco aphid, *M. persicae* and barley aphid, *M. avenae*, respectively. Duration of larval period ranged between 8.33 days on barley aphid, *M. avenae* and 11.00 days on nerium aphid, *A. nerii*. Pupal period was 4.00 days on tobacco aphid, *M. persicae* and 6.00 days on akk aphid, *A. nerii* . While the fecundity of *M. sexmaculatus* varied from 157.33 eggs on tobacco aphid, *M. persicae* to 418.00 eggs on nerium aphid, *A. nerii*.

DETRIMENTAL ROLE OF WATER POLLUTION ON GROUND SPIDER FAUNA IN WHEAT FIELDS

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Land pollution has triggered many successions in the population structure of invertebrate community in all agro-ecosystems. To underline these changes for ground

spider fauna (a potentialable bio-control agent), the present research plan was launched in wheat fields at University of Agriculture, Faisalabad (Land Utilization Farm) for the session 2008. For this purpose, two wheat fields was selected, one treated (irrigated with sewerage water) and other non-treated field (irrigated with clean tube-well water). Fortnight samples were collected through pit-fall trapping method by placing 35 jars in each one acre field which were filled with 1:1 ratio of ethyl alcohol and glycerin (200ml). The collected spiders were identified with respect to authentic keys upto species level. In total, 413 specimens were collected from both fields – 226 specimens were collected from treated field belonged to 06 families, 14 genera and 27 species, while, 187 specimens from non-treated field belonged to 05 families, 13 genera and 25 species. Family Lycosidae, Thomisida, Salticidae, Clubionidae, Oxyopidae and Araneidae were recorded in treated field, while all the families except Araneidae were present in non-treated field. Sex ratio of male was dominant in both field (203/226 in treated and 156/187 in non-treated field). Results of whole research were non-significant ($P < 0.05$) rejecting null-hypothesis that there is detrimental role of land pollution occurring due to sewerage water irrigation on the ground spider fauna. It has been concluded by these results that some elements exists in the sewerage water those are supporting factors for the proliferation of spider diversity/population. Therefore more research should be undertaken to highlight these factors for the sustainability of spider community and agro-ecosystem.

**USE OF SEX PHEROMONES AND LIGHT TRAPS TO MONITOR THE
POPULATION OF ADULT MOTHS OF COTTON BOLL WORMS IN
HYDERABAD, SINDH, PAKISTAN**

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Cotton bollworms, *Earias insulana* (Spiny bollworm), *E. vittella* (spotted bollworm) *Pectinophora gossypiella* (pink bollworm) and *Heliothis armigera* (American bollworm) are major lepidopteron pest of many crops of Pakistan, like Tomato, Chick Pea, Tobacco and many other vegetables etc but it causes serious damage to cotton crop. Sex Pheromones and Light traps are two effective techniques to control the population of adult moth. We have chosen two main cotton growing localities of Hyderabad, Sindh, Pakistan for study. Light traps and specific Sex Pheromone traps were fixed on one hector area in each locality to monitor the adult population of all species. The lures of traps were changed after the interval of 15 days and data were collected weekly. We have noted the Metrological observations. Light traps were seemed more effective than Sex pheromones in capturing the *Heliothis*, but for *Pectinophora gossypiella* Sex pheromones proved more successful than Light traps, only few could captured through Light trap, while *Earias insulana* and *Earias vittella* were captured almost in equal number by both techniques in all studying areas. Our study also revealed that the population of both

Earias species were very high throughout the year while *Heliothis* appears only in early months of season but *Pectenophora* were found in greater number during September to November almost in all localities. The population of both *Earias* species was high throughout the year, while *Heliothis* appears only in early months of season but *Pectenophora* was found in greater number during September to November.

MONITORING OF ADULT MOTHS OF COTTON BOLL WORMS THROUGH SEX PHEROMONES AND LIGHT TRAP METHOD

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Cotton bollworms, *Earias insulana* (Spiny bollworm), *E. vittella* (spotted bollworm) *Pectinophora gossypiella* (pink bollworm) and *Helicoverpa armigera* (American bollworm) are major lepidopteron pest of many crops of Pakistan, like Tomato, Chick Pea, Tobacco and many other vegetables but causes serious damage to cotton crop. Sex Pheromones and Light traps are two effective techniques to control the population of adult moth. Present study was carried out in 2008 in four cotton growing localities of Hyderabad, Sindh, Pakistan such as Hala, Saeedabad, Tando Allahyar and Jamshoro. Light traps and specific Sex Pheromone traps were fixed on one hector area in each locality to monitor the adult population of all species. The lures of traps were changed after the interval of 15 days and data were collected weekly. The metrological data was observed. During present study *Earias insulana* and *Earias vittella* were captured almost in equal number by both techniques in all studying areas and found through out the season, *Pectinophora gossypiella* mostly captured through Sex Pheromones trap. *Helicovepa armigera* was only observed in only one locality. During present Study effects of rainfall were also observed on the population of cotton bollworms. The population of *Earias species* and *Pectenophora* species was high throughout the year, while *Heliothis* appears only in Hala Rain fall effects the population

A NEW PREDATORY MITE SPECIES OF THE GENUS AGISTEMUS (STIGMAEIDAE: ACARI) FROM PUNJAB, PAKISTAN

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Mites belonging to the family stigmatidae are well known bio-control agents for the phytophagous mites and small soft bodied insects. A survey was conducted to explore

the predatory mite fauna of Punjab (Pakistan), a new record of genus *Agistemus* was collected from Rawalpindi on pear plant *Pyrus communis* and has been described in this research paper. Specimens were mounted on the glass slides with the help of Hoyer's medium. The drawings of different body parts were prepared with the help of an ocular grid in a high power microscope. These specimens were compared with the already described species. Ceremonial description, illustration of main body parts, host range and comparison remarks are also given. The other fourteen (14) paratypes were also collected from the same locality. All specimens were deposited in the Acarology Research Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan.

OCCURANCE OF THE PESTS OF MAIZE AND BARLEY CROP AT DOAABO QASIMABAD

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Pakistan is an agricultural country and cereals are considered as staple food of the people of Pakistan. Maize and barley are grown in the intermediate climate, which Pakistan has. Maize being the highest yielding cereal crop in the world is of significant importance for countries like Pakistan, where rapidly increasing population has already out stripped the available food supplies and barley is the most energy-efficient food available in nature. Ranking of barley is next to the maize. Farmers do not pay attention towards maize and barley crops because both crops are less commercial. Consequently, both crops frequently attacked by different types of pests. Presently this paper deals with systemic enumeration of maize and barley pests from four different fields at Doaabo area, Qasimabad road and both sides of national highway Jamshoro. We had started our fieldwork from Aug-Oct 2009. We visited the fields weekly where the different kinds of pests had been observed. We have collected them brought to the Lab. and identified them under dissecting microscope. We have found the Chinese grasshoppers (*Acrida cinerea*), Brown stink bug (*Acrosternum hilare*), Spotted stem borer (*Chilo partellus*), Greasy cutworm (*Agrotis ipsilon*), Chinch bug (*Blissus leucopterus*) on maize crop. While on the barley Mellon fruit fly (*Bacteriocera cucurbetea*), Hairy caterpillar (*Amsacta lactinea*), and Spotted stalk borer (*Chilo partellus*) was also found. These pests were causing major destruction to the maize and barley crops and both crops were heavily infested. Besides these pests of crops we have also found the predators in the crops like lacewings, beetles, crickets, bees etc. As we have found stalk borer in greater number, attacking on both crops and causes heavy infestation therefore we have observed their feeding behavior in the laboratory at early stage of their life cycle by rearing them in the laboratory.

**COMPARATIVE SURVIVAL OF *CHRYSOPERLA CARNEA* (STEPHENS)
(NEUROPTERA: CHRYSOPIDAE) IN COTTON WITH AND WITHOUT
INSECTICIDES UNDER NATURAL FIELD CONDITIONS**

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Cotton, *Gossypium hirsutum* L., the economy dependent crop in agricultural sector of Pakistan. Its yield for the last few years is reduced by the loss of crop due to some factors and among them, the heavy pest attacks is the most important one. Insecticide is the common practice of insect pests control, which does not meant to be the factor of increased yield. Besides environmental pollution, these insecticides also cause the destruction of naturally occurring fauna of bio-control agents. *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is one of the most important natural predator of cotton pests and the repeated use of pesticides in crops disturbing its natural equilibrium. Present field study was therefore, planned to evaluate the natural presence of *C. carnea* in cotton and a field trial under natural conditions was conducted at Rehmat-ul-Allah Tareen farm in Lodhran in year 2007. Cotton cultivar MNH-786 was planted by making two treatments each with three replicates with plant to plant and row to row distance of 76 and 30 cm. In one treatment, pesticides were used according to the need of pest management of the crop, while in other treatment no pesticide was used and this treatment separated by a heavy strip of sorghum crop. Standard agronomic practices were adopted throughout the season. Data were collected on weekly basis from each replicate of both treatments by observing eggs of *C. carnea* from five randomly selected plants. Results showed that presence of *C. carnea* eggs on pesticide free treatment was comparatively higher to that of pesticide treatment. The overall presence of *C. carnea* eggs was highest in the month of August and September while lower in June and July in both the treatments.

BIOLOGY OF CABBAGE APHID UNDER LABORATORY CONDITIONS

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Biology of cabbage aphid, *Brevicoryne brassicae* L. was studied on canola leaves under laboratory conditions at Multan, Pakistan. Biological parameters were studied by feeding canola leaves to aphid in 50 ml capacity plastic vials. Food was changed daily.

Aphid had a pre-reproductive and reproductive period of 2.34 and 6.25 days, respectively. Number of nymphs produced per female was 30.79 and reproductive rate was 3.85 nymphs per female per day. Longevity of reproductive females' was 9.0 days. Nymphs completed three instars in 9.09 days. Mean duration of first, second and third instars was 2.09, 3.50 and 3.50 days, respectively. Mortality of nymphs during development was 67.85, 17.85 and 14.30 percent in first, second and third instars, respectively. Out of the total nymphs produced 54.16 % reached the reproductive stage.

A PRELIMINARY SURVEY OF SANDFLIES (PHLEBOTOMINAE) FROM INDOOR SITES AT TEHKAL PESHAWAR (N.W.F.P)

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A survey was conducted to know the species composition of sandflies of Tehkal Peshawar from April 2008 to February 2009. Adult sandflies were collected from ten different houses. Eight species belonging to two genera were identified. Genus *Sergentomyia* was represented by six species which were *S. baghdadis*, *S. babu*, *S. dentata*, *S. fallax afghanica*, *S. tiberiadis pakistanica* and *S. christophersi* while genus *Phlebotomus* was represented by two species, *Ph. papatasi* and *Ph. salehi*. Among 698 Sandflies, *S. baghdadis* was found to be the most abundant one, as 40.97% of the total collection belongs to this species and was recorded in most of the study period. *S. babu* was the next abundant species represented by 29.08% in the collection. *S. dentata* was represented by 17.62, *S. fallax afghanica* by 7.59, and *Ph. papatasi* by 4.29%, while the other three species, *S. tiberiadis pakistanica* and *S. christophersi* and *Ph. salehi* were represented by only two individuals each. *S. baghdadis*, *S. babu* and *S. dentata* were recorded from all the houses. *S. fallax* and *Ph. papatasi* both were recorded from eight houses. Rests of the three species were recorded from only one or two houses.

PREDATORY EFFICACY OF LADYBIRD BEETLE, *COCCINELLA SEPTEMPUNCTATA* (L.) ON CABBAGE APHID, *BREVICORYNE BRASSICAE* L. AND TURNIP APHID, *LIPAPHIS ERYSIMI* (KALT.) UNDER LABORATORY CONDITIONS

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Predatory efficacy of larvae and adults of ladybird beetle, *Coccinella septempunctata* (L.) was investigated under laboratory conditions at room temperature

during months of February-March at Dera Ghazi Khan during 2006 on cabbage aphid, *Brevicoryne brassicae* L. and turnip aphid, *Lipaphis erysimi* (Kalt.). Twenty five nymphs of both the species were collected from field grown crop of *Brassica napus* L. and put in a petri dish of 5 cm diameter. Experiment was replicated three times. In each replication nymphs were offered in 10 petri dishes to a larva or an adult of *C. septempunctata*. Mean numbers of nymphs consumed daily were counted. *C. septempunctata* consumed 4.93, 6.96, 11.21, 11.70 & 15.60 and 5.5, 7.26, 10.85, 12.9 & 15.78 nymphs of *B. brassicae* and *L. erysimi* in 1st instar, 2nd instar, 3rd instar, 4th instar and adult stage, respectively.

ACTIVITY AND BIOLOGICAL EFFECT OF *NEPETA CLARIKIE* HOOK. F. (LABIATAE) AGAINST INSECTS OF MEDICAL AND VETERINARY IMPORTANCE

SYED ISHTIAQ ANJUM, MUHAMMAD ISMAIL, ABDUSSALAM, JEHANGEER KHAN, NAEEM KHAN, SULTAN AYAZ, JAVID HUSSAIN AND MUSSARAT YOUSUF

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To assess the larvicidal efficacy of a *Nepeta clarkei* Hook. f. (Labiatae) aqueous extract on *Culex* (Diptera/Culicidae), larvae. Fourth instars larvae were exposed as a normal media supplemented with *Nepeta clarkei* formulations in different concentration (0.025% 0.05%, 0.1%, 0.15%, and 0.2%). A control group of larvae was exposed to an aquatic media without extract, reported non-significant mortality. The lethal concentration at which 50% of larvae failed to become pupate and died was found to be 0.1% at 24 hours of exposure. Significant reductions on growth and pupation, besides prolonged larval periods, were observed in treated and compared with control. The chloroform extract showed 100% mortality when applied for solvent toxicity in contrast, produced no growth disruption within the tested range of concentrations.

SPECIES COMPOSITION OF MOSQUITOES IN TEMPORARY HABITATS IN UNIVERSITY OF PESHAWAR CAMPUS

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This study was carried out to know species composition of mosquitoes in temporary habitats. Six temporary habitats including bamboo glasses, discarded containers, pond water, mud pots and tyres at Peshawar University Campus were used for

the collection of immature stages of mosquitoes. A total of 2419 adult mosquitoes (1433 females and 985 males) comprising six species viz. *Ae. albopictus* (57.2%), *Ae. unilineatus* (2.5%), *Ae. walbus* (2.8%), *Ar. subalbatus* (3.8%), *Cx. Quinquefasciatus* (33.6%) and one *An. stephensi* belonging to four genera were recovered from all studied habitats. Among the total collected mosquitoes *Ae. albopictus* was the most frequently found species during the studied period and was recorded from four habitats including bamboo glasses, discarded containers, waste water and mud pots. Among the rest studied species *Cx. Quinquefasciatus* was the second most abundant species collected from three habitats viz. waste water, pond water and mud pots; *Ae. unilineatus* and *Ar. subalbatus* was recorded from two habitats, bamboo glasses and discarded containers. While *Ae. walbus* was recorded only from bamboo glasses. Only one mosquito larva of *An. stephensi* was collected from waste water.

**BIOLOGICAL ACTIVITY OF FLUFENOXURON, A CHITIN SYNTHESIS
INHIBITOR, ON VARIOUS STAGES OF RED FLOUR BEETLE DUE TO
LARVAL FEEDING ON FLUFENOXURON TREATED-DIET**

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Red flour beetle is an economically important pest of stored grain commodities through out the world. Flufenoxuron, a chitin synthesis inhibitor, was tested against two strains of *T. castaneum* at concentrations of 0.006, 0.003, and 0.0015 ppm by exposing 15-day old larvae of *T. castaneum* to flufenoxuron treated wheat flour under laboratory conditions. Flufenoxuron caused significant effects on larval mortality, larval weight, larval period, pupation, adult emergence and finally on next progeny at all the concentrations against both the strains. Flufenoxuron caused 17.86% and 16.07% mortality of larvae at 0.006 ppm after 5 days of treatment for Multan and Faisalabad strains respectively. Similarly at 0.006 ppm larval weight was reduced by 8.76% and 8.88% of Multan and Faisalabad strains respectively. Larval period was prolonged significantly at all the concentration for both the strains. Pupation was severely affected, resulting in inhibition of 75.20% and 70.83% (at 0.006 ppm) for Multan and Faisalabad strains respectively. Emergence of adults was inhibited by 39.11% and 47.67% at 0.006 ppm for Multan and Faisalabad strains respectively. Growth inhibitory effects were continued to the next generation due to the transovarial activity of flufenoxuron, causing 94.19 and 97.11 percent reduction (at 0.006 ppm) in F1 adult population of Multan and Faisalabad strains respectively. Present results show the potential usefulness of flufenoxuron in storage houses and godowns for the management of red flour beetle and other stored grain pests.

MANAGEMENT OF TERMITES AND OTHER SOIL PESTS IN GROUNDNUT

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Strategies for the management of Termites and other Soil Pests in groundnut were developed at the National Agricultural Research Centre (NARC), Islamabad using different composts viz., (neem, chicken farm yard manure, sheep farm yard manure, farm yard manure, leaf manure and biokhad) and soil solarization using transparent and different coloured polyethylene sheets. In trials for the control of soil pests, particularly of termites in groundnut at NARC, insecticides were also used included chlorpyrifos, isofenphos granules, chlorpyrifos, phorate, carbosulfan and carbofuran in controlled release formulations, and chlorpyrifos seed dressing. Their effects on foliar pests were also noted. Observations revealed that termites were significantly reduced (77.2%) in treatments applied with neem in combination with soil solarization using transparent polyethylene sheets in comparison with the insecticidal trials (21.7%). These results could be used in the future studies for integrated disease management in field crops.

DIFFERENT COMPOSTS AND RESISTANT GENOTYPES FOR THE MANAGEMENT OF STEM BORER *CHILO PARTELLUS* IN CORN

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ifferent composts and resistant genotypes were used for the management of stem borer (*Chilo partellus*) in corn at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. Experiments conducted with different composts (farm yard manure, chicken farm yard manure, neem leaves and biokhad) showed that neem was the best in controlling ear rot diseases (caused by *Aspergillus flavus* and *Fusarium moniliforme*) as well as stem borer., *C. partellus* () significantly. Experiments conducted with different corn genotypes viz., Local-Y, Local-W, EV-1097, Margalla, Rakaposhi, Soan-3, NRL-4, NRL-6, NCML-73, SP-3, NC-2003, NC-2703 revealed that Soan-3 followed by Rakaposhi was the best genotype in controlling the above ear rot diseases (7.6 and 10.2%) along with reducing the incidence of *C. partellus* (11.2%) and ultimately increasing the grain yield (5936 and 4688 kg ha⁻¹).

SECTION - III

ENTOMOLOGY

PYGMY GRASSHOPPERS (TETRIGIDAE) OF SINDH

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The genus *Criotettix* Bolivar is added to the previously reported *Ergatettix* Kirby, *Hedotettix* Bolivar, and *Paratettix* Bolivar. The specimens are closely related to *Criotettix latifrons* Hbard and collected from desert area of district Sanghar. The distribution is considered at Pakistan – India Sub continent.

MORPHOLOGICAL AND BEHAVIOR DIFFERENCES BETWEEN GAINT HONEY BEE (*APIS DORSATA*) AND DWARF HONEY BEE (*APIS FLORAE*)

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There were two wild species of honey bee, *Apis dorsata* and *Apis florea* studied in hilly and plane area of Betanni, the South West of NWFP Pakistan. *Apis dorsata* is giant honey bee while *Apis florea* are dwarf honey bee. The difference of all organs was found as, body length 8.35 mm, thorax length 1.28 mm, abdomen length 4.20 mm, antennae length 0.78 mm, forelegs length 3.15 mm, mid legs length 4.64 mm, hind legs length 7.89 mm, forewing length 6.46 mm, hind wings length 3.21 mm. In hilly area, *Apis florea* found active in July and August because of the temperate weather giving more honey and reproduce. They produce up to three kg honey in this season. The nuptial flight was also found at dusk time. But in September-October and January-February they found very passive due to low temperature. In plane area *Apis florea* found active in September and October due to normal weather. They produce more honey and reproduce. But in July-August found less active due to high temperature and in January-February due to low temperature. *Apis florea* were found in solitary form having no group behavior. The height of nests found 3 to 4 meters in small trees and shrubs. In *Apis florea*, there is no flight found in night out side the nest but when light fall on the hive then bees come out towards the light. In hilly area *Apis dorsata* was found negligibly due to low temperature, irregular mountains and small trees. In hilly area, the colonies of *Apis dorsata* was also found in less number due to high temperature and less availability of flowers in July-August but in September and October they immigrate from other areas and large number of colonies were found in the form of groups. They have colonies group behaviors. In this

season *Apis dorsata* reproduce and there were three colonies found in nuptial flight. The night fight was also found in *Apis dorsata* at 11:45 PM, 11:33 PM and 12:05 AM. It was also found statistically that there is a significant difference ($p = 0.005$) in the behavior and morphology of both types of honey bees

COMPARISON OF PREY QUALITY, MONOTYPIC DIETS: EFFECT ON FITNESS, GROWTH AND SURVIVORSHIP OF FEW SPIDERS REARED IN LABORATORY

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Information on the value and the influence of various natural diets such as aphids (control), jassid, house and fruit fly, citrus leaf minor, and mosquito larvae (treatments) were individually tested for growth and survivorship against eight most common spiders, *Pardosa birmanica* Simon 1884, *Pardosa oakleyi* Gravely 1924, *Lycosa wroughtoni* Pocock 1899, *Hippasa maduhae* Tikader & Malhotra 1980, *Hippasa partita* (Cambridge) 1875, *Plexippus paykulli* Savigny 1825 (cursorial), *Marpissa bengalensis* Tikader 1974 and *Philodromus assemensis* Tikader 1962 (foliar) and classified the quality of food types for survivor, growth and development. Spider lings of each species were reared on monotypic diets from 3rd molting to maturity through adulthood on selected diets at 27°C, 80% RH, 12:12 hrs. LD periods. Fitness was calculated from survivorship and life span, life expectancy by the time period they survived in laboratory after adulthood. Individually all these diets did not fulfill the nutritional requirement and spider lings survived only few developmental stages. Only in three cases spider ling reached maturity but reproductive success was zero. Only two preys proved to be of slightly of better quality, showed significantly higher survival rate as compared to other diets. These results indicate that there are fitness related consequences of prey specialization.

DOES THE LAND USE TYPES AFFECT POLLINATOR DIVERSITY AND REPRODUCTIVE SUCCESS IN CLOVER (*TRIFOLIUM ALEXANDRINUM* L.)?

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Investigations to identify the effect of specific land use types on pollinator diversity and reproductive success in Egyptian clover (*Trifolium alexandrinum* L.) were

carried out in three topographically distinct locations (semi-natural dessert habitat, intensive agriculture land and hill torrent area) at district D.G. Khan, Pakistan. Plants were grown in pots and hundred pots were kept at each location. The Egyptian clover is a self-pollinated autogamous forage crop but for better seed setting tripping agencies (pollinators) are required. Pollinator fauna of Egyptian clover was composed of Bees, wasps, butterflies, moths and true flies. Diversity Indices (ShannonWiener and Hulbert indices) revealed semi-natural dessert habitat as the best in terms of pollinator abundance and diversity. Bees were the dominant floral visitors in semi-natural desert habitat, with *Apis dorsata*, *A. florea* and *Thyreus* sp. being the most abundant, followed by the butterflies. Visitation rates also confirmed the relative effectiveness of bees in all three locations. Semi-natural dessert habitat also proved to be the best in terms of number of seeds, seed weight per head and germination percentage. Although, the remaining two locations were statistically non-significant with respect to yield attributing parameters but were significantly better than that of control. The Pearson correlation between number of seeds and seed weight/head revealed a significant positive (0.95) relationship. Since the high bee diversity resulted in maximum seed set of clover in semi-natural dessert habitat, it is concluded that conserving these bee species may enhance clover production in Pakistan.

**ON THE FINDING OF *SCELIO HIEROGLYPHI* TIMBERLAKE FROM
PAKISTAN (HYMENOPTERA: PROCTOTROPOTIDEA: SCELIONIDAE)**

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Scelio hieroglyphi Timb. egg-pod's parasite of *Hieroglyphus oryzivorus* Carl is being reported for the first time from Pakistan. Infestation percentage was recorded 23.67%. As predicted, total and initial development of eggs decreased in parasitized egg-pods of *H. oryzivorus*. The present study showed that the effect of parasitism on development of eggs could have a large impact on grasshopper population dynamic.

**A PRELIMINARY STUDIES ON THE GENUS *ACROTYLUS* (OEDIPODINAE:
ACRIDIDAE:ACRIDOIDEA:ORTHOPTERA) FROM PAKISTAN**

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About 58 specimen of the genus *Acrotylus* namely, *Ahumbertians* ,Saussure, *Ainsubricus insubricus*, Scopoli, *Ainsubricus inficitus*, Walker, *Alongipes longipes*, Charpentier, *A longipes subfasciatus*, Bei-Bienko, has been collected from the various

Provinces of Pakistan. Some species male and female genitalia is studied for the first time. The distribution of *Acrotylus* grasshoppers in various provinces of Pakistan is also considered.

**A NEW PREDATORY MITE SPECIES OF THE GENUS *PSEUDOSTIGMAEUS*
(STIGMAEIDAE: ACARI) FROM PUNJAB, PAKISTAN**

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MUHAMMAD KAMRAN AND ABUBAKAR RAZA

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Mites belonging to the family stigmatidae are known predators against the phytophagous mites and small soft bodied insects. A survey was conducted to explore the predatory mite fauna of Punjab (Pakistan), a new species of genus *Pseudostigmaeus* was collected from Jhang on Lehli weed (*Convolvulus arvensis*) and has been described in this research paper. Specimens were mounted on the glass slides with the help of Hoyer's medium. The drawings of different body parts were made with the help of an ocular grid in a high power microscope. These specimens were compared with the already described species. Ceremonial description, illustration of main body parts, host range and comparison remarks are also given. Seven (7) paratypes were collected from the same collection data and six (6) were collected from Khanewal on cotton crop. All specimens were deposited in the Acarology Research Laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan.

**BIODIVERSITY AND RELATIVE ABUNDANCE OF SPIDER SPECIES
RESIDING IN RICE AGRO-ECOSYSTEM AT FAISALABAD**

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A survey of spiders associated with rice fields at the University of Agriculture, Faisalabad, Pakistan was conducted through August 1996 to December 1996. During five months 100 Pit fall traps were operated for 5 consecutive days. A total of 1937 individuals belonging to eight families, 18 genera and 37 species were recorded. Maximum number of spiders was captured in September and August. In September spider population increased and after that number of spiders in the sample continuously declined. However, the size of monthly samples was different at a statistically significant

level ($F = 52.7$ d.f = 4, 0.05). *P. birmanica*, *P. sumatrana*, *P. sp1*, *P. songosa* and *Oxyopes* were considered as dominant species. Maximum diversity was recorded in August (2.791) and it was minimum in November (0.9864). A significant variation in the monthlu sample of evenness indices was recorded. August (2.20) and November (1.265) was lower but it increased in September (2.542), October (2.54) and December (2.132).

PHYLOGENETIC ANALYSIS OF SOME COMMONLY FOUND SPIDER SPECIES IN WHEAT AGRO-ECOSYSTEM AT FAISALABAD

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Knowledge about the systematic research has been seriously limited by the poor information on spiders which lack many clear cut diagnostic characters. This problem can be inferred by a phylogenetic studies based on DNA sequences of mitochondrial cytochrome oxidase sub-unit 1. DNA barcoding identification system represents a promising approach to resolve this taxonomic problem. This relies on amplifying and sequencing a short DNA fragment and comparing this to the reference library for identification of species. Cytochrome oxidase 1 (COX1) is proved to be a suitable marker for spider species identification. This project is planed to study the species diversification of a guild of spiders residing in wheat agro-ecosystem of Faisalabad through DNA barcoding.

DETERMINATION OF GENETIC DIVERSITY AND PREDATOR-PREY RELATIONSHIP IN SOME SELECTED ARTHROPOD SPECIES BY USING RAPD TECHNIQUES

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Genetic diversity of twelve arthropod species viz. *Coccinella septumpunctata*, *Cheilomenes sexmaculata*, *Hippodemia convergens*, *Hippodemia variegata*, *Chrysoperla carnea*, *Oxyopes javanus*, *Neoscona theisi*, *Macrosiphum miscanthi*, *Schizaphis graminum*, *Aphis. maidis*, *Bemisia tabacii* and *Musca domestica* was accessed by RAPD markers. A total of 195 fragmnets were amplified by using 25 RAPD primers. Out of which 182 fragments were polymorphic showing 93% polymorphism. The number of

amplification products varied between 6 to 10 with an average of 7.08 per primer. Genetic characterization was done with the help of cluster analysis constructed on the basis of similarity matrix. Three main cluster groups were depicted. In one group only (herbivore) prey species were present. Second group contained all (carnivore) predator species. Among them arachnids were in separate cluster from the rest of predator species. A single band of approximately 900bp was identified in DNA sample of *Coccinella septumpunctata*. Similarly another band of approximately 750bp was identified in DNA sample of *Macrosiphum miscanthi*. These fragments could be used as fingerprints for the identification of *C. septumpunctata* and *M. miscanthi*. Predator-prey relationship was observed on the basis of comparison among control, fed predator and preys. Few unique fragments of preys were identified in the fed predators, suggesting a trophic link between predator and prey species studied.

**RECORD OF TWO NEW SPECIES OF THE *PHYTOSEIUS* (*PHYTOSEIUS*)
RIBAGA (*PHYTOSEIIDAE*: *ACARI*) FROM NORTHERN AREAS OF
PAKISTAN**

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Mites belonging to the family Phytoseiidae are known bio-control agents for the phytophagous mites and small soft bodied insects. A survey was conducted for collection of Phytoseiid mites different localities of Pakistan which resulted in collection of two new specie *Phytoseius* (*Phytoseius*) *kenos* and *Phytoseius* (*Phytoseius*) *caulis* from the northern areas. *P. kenos* was collected from Hunza valley from Apricot (*Prunus armeniaca*) and *P. caulis* was collected from Muzaffarabad (Azad Kashmir) from Pear (*Pyrus communis*). Holotypes were deposited in the Acarology Research Laboratory, Deptt. of Agri. Entomology, University of Agriculture, Faisalabad. Specimens were mounted on the glass slides with the help of Hoyer's medium. The drawings of different body parts were prepared with the help of an ocular grid in a high power microscope. These specimens were compared with the already described species. Ceremonial description, illustration of main body parts, host range and comparison remarks are also given.

POLLINATOR COMMUNITY OF SUNFLOWER (*HELIANTHUS ANNUS*) AND ITS ROLE IN CROP REPRODUCTIVE SUCCESS

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Pakistan is self sufficient only in one third of the requirements for edible oil while the remaining requirement is met by import. In Pakistan, sunflower is the second most important source of oil after cotton and contributes about 32% to domestic production of edible oil. Therefore, sunflower needs special attention among the oil seed crops. Following study was designed to identify the pollinator community and its role in reproductive success of sunflower. The experiments were carried out at research farm of University College of Agriculture, Bahauddin Zakariya University, Multan. The community of pollinators was composed of 14 insect species that included 8 bee species (Hymenoptera), 4 true fly species (Diptera) and 2 butterfly species (Lepidoptera). Bees were the most frequent floral visitors in sunflower. *Apis dorsata*, *Xylocopa* sp. and *Megachill* sp. were most abundant among the bees and their visitation frequencies per flower head were also highest i.e. 1.33, 0.07 and 0.06 respectively. Family Syrphidae proved most effective among the rest of floral visitors in terms of abundance with *Eristalinus aeneus* and *E. arvorum* were most abundant. The peak visitation activity of pollinators was recorded between 12:00 to 14:00 hr. The yield attributing components revealed the significance of insect pollination in sunflower as the weight of the head, number of seeds, seed weight and germination percentage was better under open pollination treatment compared to caged pollinated plants. Moreover, a high percentage of abnormal seeds in caged pollinated plants further validated the importance of insect pollination in sunflower.

POPULATION FLUCTUATION OF FRUIT FLIES FROM DIFFERENT HOST FIELD PLANTS IN SARGODHA REGION, PAKISTAN

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The aim of the research work was to determine the population's fluctuation of fruit flies in different seasons in different crops and to study the effect of abiotic factors (temperature and humidity) on the population fluctuation of male fruit fly. Methy

euginol traps were used in three seasons (autumn, winter and spring) on three different crops (citrus, guava and vegetables). The traps were placed in three different fields from September 2008 to March 2009. The data was collected after 7 days interval in different environmental conditions. Different seasons and crops showed the significant correlation with the population fluctuation of male fruit flies. Vegetables have higher population fluctuation of male fruit flies (11.639) followed by the citrus (7.750) and guava (7.694). Population of male fruit flies was increased in autumn and decreased in winter. The negative correlation was noticed between humidity and temperature as well as between humidity and population, but the population of male fruit flies and temperature were positively correlated.

BIODIVERSITY THREATS TO INSECTS ASSOCIATED WITH RICE CROP IN THE PUNJAB, PAKISTAN

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During a survey through face to face interviews of farmers mal-farming practices of rice farmers as threat to insect biodiversity associated with rice crop agroecosystem were investigated. Excessive use of agrochemicals and rice straw burning along with animal grazing were explored as major threats to insect biodiversity of this man made wet land. Rational use of agrochemicals and legislations about banning of rice straw burning and cattle grazing in harvested rice fields were proposed.

COLEOPTEROUS INSECT FAUNA ASSOCIATED WITH RICE CROP AGROECOSYSTEM OF THE PUNJAB, PAKISTAN

MUHAMMAD ASGHAR, ANJUM SUHAIL, ARSHED MAKHDOOM SABIR AND MUHAMAD ARSHAD

**Pest Warning & Quality Control of Pesticides, Fairozewala, Lahore, Pakistan (MA), Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan (AS, MA), and Rice Research institute, Kala Shah Kaku, Pakistan (MS)*

The study of the biodiversity of coleopterous insects associated with rice crop agroecosystem in the Kallar tract showed 145 species having various kind of association with rice crop as pest, predator, visitors and scavengers. Scarabaeidae, Staphylinidae, Hydrophilidae, Dytiscidae, Carabidae and Anthicidae were major ones followed by Elateridae, Coccinellidae, Scydmaenidae, Chrysomelidae, Erotylidae, Curculionidae,

Latridiidae, Dascillidae, Heteroceridae, Nitidulidae, Tenebrionidae, Pselaphidae, Cicindelidae, Lagriidae, Geotrupidae, Scolytidae, Meloidae, Cerambycidae, Bostrichidae, Helophoridae, Lampyridae and Hydraenidae. The details about biodiversity had been arranged that can serve as formulating principals for any IPM strategy in the rice crop agroecosystem. The values of species abundance, Shannon's, Simpsons's and dominance for Coleoptera were 260694, 3.0824, 0.0962 and 0.6194, respectively.

CAN COMPETING SPIDERS CO-EXIT IN THE SAME HABITAT?

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Tetragnatha javana, *Leucauge decorata*, *Neoscona theisi* and *Argeope pradhani* are four common orb web spiders of rice ecosystems of central Punjab, Pakistan. In spite of apparent competition among these spiders for resources they co-exist in the same habitat. Present study was designed to investigate the possible factors which make their co-existence possible. Results of our study showed that orb web spiders consumed same prey orders but in different proportions. Four orb web spiders also differed in their reproductive strategies and in the prey size selection. Niche overlap values indicated that each of the four orb web spiders was utilizing the time, habitat and prey resources differently. Discriminate function analysis also clearly separated the four species in three-dimensional space. It is concluded that differently utilization of time, microhabitat and prey resources reduce competition among these spiders and make their co-existence possible. Co-existence of predator species whose niche axis varies in the same habitat enhances their biocontrol potential.

FECUNDITY AND DEVELOPMENT OF COWPEA BEETLE, *CALLOSOBRUCHUS CHINENSIS* ON PULSES

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Pulses are a good source of food nutrients, proteins, carbohydrates, lipids, vitamins and minerals which are the essential part of every Pakistani's diet. After harvesting pulses are stored as grains and these are attacked by a number of stored grain pests. The cowpea beetle, *Callosobruchus chinensis* is one of the important insect pests attacking the pulses and reducing the quantity and quality of its host grains. The

fecundity and development of *Callosobruchus chinensis* (Coleoptera: Bruchidae) on six different pulses grains *i.e.*, whole black gram, white gram (crushed and whole), lentil, mung bean, red cowpea and white cowpea were studied under controlled conditions of $30 \pm 4^\circ\text{C}$ and RH $40 \pm 5\%$. The highest fecundity of *C. chinensis* was recorded on whole black gram averaging 54.4 eggs/ female while lowest on the white crushed gram and lentil averaging 27.0 eggs/ female were recorded. The total development time of egg-adult was the longest on lentil *i.e.*, 18 days and it varies from 14-16 days for other hosts. Among the pulses which we evaluated here lentil, white cowpea and whole black gram are favorable hosts for the development of cowpea with more than 80 % adult emergence rate. While it failed to complete life cycle on the red cowpea and cereals *i.e.*, wheat, rice and maize and all the grubs died before pupal stage. It suggested that red cowpea and these cereals were not favorable hosts for the development of cowpea beetle, *Callosobruchus chinensis*.

EFFECT OF ADJACENT CROPS AND AGRONOMIC PRACTICES ON THE ACTIVE DENSITY AND DIVERSITY OF SPIDERS IN WHEAT ECOSYSTEMS

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Present study was aimed at to describe the effects of five wheat habitats (which differed in adjacent crops and agronomic practices) on the activity density, richness, evenness, diversity, guild structure of spiders and pest populations. The study was conducted in district, Sheikhpura Punjab, Pakistan during 2005-6 and 2006-7. A total of 23097 specimens of spiders belonging to 47 species, 31 genera and 12 families were collected from five different habitats. Overall diversity and evenness of spiders did not differ among different habitats; however abundance and richness was significantly different. All sampled habitats had similar family and species composition. Significant positive correlation was observed between active density of agrobiont spiders and prey populations.

A REVISION OF MYROCHEINE GENUS *ENNIUS* STÅL WITH SPECIAL REFERENCE TO REDESCRIPTION OF *E. MONTEIRONIS* DISTANT (HEMIPTERA: PENTATOMIDAE: PENTATOMINAE)

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Ahmad and Zahid (2007) described a new species of *Ennius* Stål *i.e.* *E. neomorio* from Nigeria, with special reference to its metathoracic scent auricle and male genitalia.

Linnavuori (1982) listed *ater* Dallas, *longulus* Germar, *monteironis* Distant and *morio* Dallas and catalogued by Riderin an unpublished catalogue (2004). Linnavuori (1982) revised the genus *Ennius* and gave a key of three specie and some illustrations of above three species but he did not completely describe anyone of them. To fill these gaps presently we give a key of all species of the genus and redescribe *E. monteironis* with special reference to characters of metathoracic scent auricle and male genitalia and in this light their phylogenetic relationship are also briefly discussed. We followed the techniques of Ahmad (1986) and ahmad and McPherson (1990 and 1998) for the inflation of aedeagus. For measurements, description and illustrations those of Ahmad and Afzal (1989).

**BIODIVERSITY OF GENUS *AMBLYSEIUS* (ACARINA: PHYTOSEIIDAE) IN
DIFFERENT AGRO-ECOLOGICAL ZONES OF PUNJAB, PAKISTAN**

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Mites of genus *Amblyseius* are well known for their predatory potential against different phytophagous mites, small soft bodied insects and their eggs. The present study was conducted to determine the distribution of different species of genus *Amblyseius* in different agro-ecological zones of Punjab. Mite specimens were collected from different sources by sieve collection and Berlese's Funnel methods during 2005-06. The collected mite specimens were deposited in Acarology Research Laboratory Department of Agri. Entomology, University of Agriculture, Faisalabad. Mites of genus *Amblyseius* were sorted from the bulk collection and identified upto species level with the help of taxonomic keys and literature published. Biodiversity of mites was determined with the help of Shannon Diversity Index. On the basis of analysis, it was concluded that no population was recorded during the course of survey from arid and Sulemania Mountainous zones in 2005-2006 except August September- 2005. Central Mixed zone, Cotton Zone and Rice zone showed maximum diversity as whole during the course of survey except during August September 2005 in which High Rainfall zone was the leading one showing maximum diversity ($H' = 0.87$). High Rainfall zone showed minimum diversity during the remaining seasons. So, results shown during August September 2005 are quite fluctuating. Remaining all the zones showed more or less intermediate diversity index values. Central mixed zone, Cotton zone and Rice zone has become the hot zone for *Amblyseius* mite population harboring maximum species richness and evenness.

**A GENERIC REVIEW OF MYROCHEINE SUBCLADE OF *LAPRIUS* STÅL
(HEMIPTERA: PENTATOMIDAE: PENTATOMINAE) WITH REFERENCE TO
THEIR PHYLOGENY**

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Ahmad and afzal (1989) redescribed the genera *Laprius* Stål, *Dollangiana* Ahmad and Kamaluddin and *Neodorpius* Ahmad and Afzal with their representative species with reference to their metathoracic scent auricle and male and female genitalia. Ahmad *et.al.*, (1996) described their new genus *Lodosia* to accommodate the only Palaearctic myrocheine species *L. gonocoxa* from Turkey in the west. Showing its relationship with *Laprius* Stål. Presently the subclade in the tribe Myrocheini *i.e.* *Laprius* Stål is reviewed with special reference to all the described species of the above listed genera and their species and their male and female genitalia and in this light their cladistic analysis is presented. The genus *Pretorius* Distant appears to be its outgroup. We followed the techniques of Ahmad (1986) and ahmad and McPherson (1990 and 1998) for the inflation of aedeagus. For measurements, description and illustrations those of Ahmad and Afzal (1989).

**FIRST RECORD OF SUGENUS *ERYTHRAEUS* (ERTHRAEUS:
ERYTHRAEIDAE) FROM PUNJAB, PAKISTAN**

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Latreille (1806) erected genus *Erythraeus* (Erythraeidae) and designated *Acarus phalangoides* (adult) deGeer, 1778 by original designation as its type species. Southcott (1995) categorized genus *Erythraeus* into two subgenera viz *Zaracarus* and *Erythraeus* and designated *Erythraeus* (*Zaracarus*) *lanciftr* as type species of subgenus *Zaracarus*. In subgenus *Erythraeus* 28 species are known from Europe and Asia and have been described both from adults and larvae. Adults and nymphs of subgenus *Erythraeus* live freely and feed on phytophagous mites, small insect pests like thrips, aphids, plant hoppers, mealy bugs etc., their eggs and eggs of many lepidopteran insect pests. Larvae of this genus parasitize different insects *i.e.* bugs and aphids etc. or live freely on different plants. As a result of survey that was carried out in different ecological zones of Punjab, Pakistan, a species of subgenus *Erythraeus* (*Erythraeus*, Erythraeidae) was

collected from *Sorghum hale pence* and *Cynodon dactylon* from Faisalabad and Rajanpur cities respectively. This pakistani species has been described in detail, illustrated along with diagrams and has been compared with closely related species. This species belongs to the species group with basifemoral setal formula 2-2-2 and short anterior sensillae. This group includes three species viz. *E. (E.) adrastus* Southcott, 1961 from Denmark, *E. (E.) tinae* Haitlinger, 1997 from Canary Islands, Tenerife, *E. (E.) picaforticus* Haitlinger, 2002 from Balearic Islands, Mallorca (Southcott, 1961, Haitlinger, 1997, Haitlinger, 2002) This species differs from *E. (E.) adrastus* in ill (32 vs. 36), tV (10 vs. 12), stemalae (1a) (50 vs. 89), IP (2230 vs. 1805), legIII (843 vs. 680), Width of scutum (W) (117 vs. 141-167), PW (75 vs. 105-118) and PSE (smooth vs. nude); from *E. (E.) tinae* in ill (32 vs. 47), tV (10 vs. 15), IP (2230 vs. 3756), TiIII (250 vs. 301), GL (120 vs. 182), ISD (60 vs. 76), Coxala I 75 vs. 128) and DS (33-42 vs. 70-130); from *E. (E.) picaforticus* in ill (32 vs. 72), tV (10 vs. 24), IP (2230 vs. 3034), GL (120 vs. 174), DS (33-42 vs. 70-76), legIII (843 vs. 1186), W (117 vs 190), PL (52 vs. 84) and Coxala I (75 vs. 114).

**A NEW SPECIES OF GENUS *SALIXOCORIS* AHMAD AND ABBASI
(HETEROPTERA: PENTATOMIDAE: HALYINI) FROM THATTA, SINDH,
PAKISTAN**

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A new species of genus *Salixocoris* Ahmad and Abbasi is described on the bases of metathoracic scent gland and male and female genitalia from Thatta, Sindh, Pakistan and compared with its closely allied species.

**WEB CHARACTERISTICS AND BODY MEASURES OF *ARGIOPE PRADHANI*
(ARANEAE: ARANEIDAE)**

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Present study was conducted to investigate the relationships between web characteristics and body measures of *Argiope pradhani* (Araneae: Araneidae). For this purpose ~ rice field (three hectare area) in Sheikupura was selected. The data of spider's webs and prey was collected in October & November 2009. Most of the spiders constructed inclined webs at the height that ranged from 35 cm to 105 cm above ground. Principal component analysis (PCA) was used for the separation of spiders on the basis of web characteristics and body measures. Most of the prey items recorded from the webs belonged to Diptera, Homoptera and Lepidoptera. Prey types and their number collected

from the webs of different heights varied significantly. For prey capture *A. pradhani* constructs webs of different sizes and at different heights.

GRASSHOPPER SPECIES COMPOSITION IN MIRPUR DIVISION OF AZAD JAMMU AND KASHMIR, PAKISTAN

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The present study represents the grasshopper species composition, diversity, abundance and richness of Mirpur division of Azad Jammu and Kashmir, Pakistan. A total number of 25 species were collected and identified from the study site. Eighteen species were found in each of Mirpur and Bhimber district while 14 species were found in Kotli district. Species diversity was highest in Mirpur followed by Bhimber and Kotli while grasshopper abundance was higher in Bhimber followed by Mirpur and Kotli. On the basis of number of specimens collected, *Oxya hyla hyla* was found most abundant in Bhimber while *Spathosternum presiniferum presiniferum* was found most abundant both in Mirpur and Kotli. In relation to species distribution pattern 9 species were broadly, 7 intermediately and 9 narrowly distributed in the study area.

BIODIVERSITY OF STAPHYLINIDS OF PUNJAB, PAKISTAN

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Staphylinids (rove beetles) belong to the largest family of beetles in the order Coleoptera of class Insecta. Because of their cosmopolitan distribution with broad latitudinal ranges, they are known to play important role as pollinators, decomposers and scavengers. The studies on their biodiversity including the species richness, distributional patterns and seasonal abundance are unknown with respect to their role in ecological system in Punjab, Pakistan. The present study therefore, was carried out to study the biodiversity of staphylinids in different ecological regions of Punjab during 2008 and 2009. The population was collected by sifting of leaf litter, FIT, pit fall, hand collection and light traps from cultivated and non-cultivated (forest) areas. The collected specimens were identified up to species level. The population build up during different times of the year with respect to their habitat was recorded. All the inter-related factors associated with the population were also recorded such as temperature, relative humidity, latitude, longitude, height from sea level, soil moisture contents and habitat specificity and their relation with population was also studied. A positive co relation was found between soil moisture contents, rainy season and staphylinids population.

**EFFECT OF IRON FORTIFIED WHEAT FLOUR ON THE BIOLOGY AND
PHYSIOLOGY OF RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*
(HERBST) (TENEBRIONIDAE: COLEOPTERA)**

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Fortification of food is being practiced in many countries to fight against the deficiencies of micronutrients spread throughout the world. Along with the benefits to human beings and effects on other animals like rats, this fortification can also influence the physiology and life stages of insects. Presents study was carried out to evaluate the effect of different concentrations of iron in fortified flour, as well as the effect of commercially available iron fortified flour, on biology and gut enzymes of *Tribolium castaneum* (Herbst)(Tenebrionidae: Coleoptera). Varying levels of iron showed significant difference in the amylase activity and protease activity as compared to control. The different quantity of iron in flour also affected the number of eggs laid, larval survival, larval weight, percentage weight loss and larval duration, however, there was no prominent variability among treatments in relation to pupal survival and pupal duration.

**INFLUENCE OF PLANT EXTRACTS ON THE LIFE HISTORY AND
POPULATION DEVELOPMENT OF HOUSE FLY *MUSCA DOMESTICA* L.
(DIPTERA: MUSCIDAE)**

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The present studies examined the use of plant extracts viz., Niaz bo (*Ocimum basilicum* L.), Gardenia (*Gardenia jasminoides* (Ellis), Snatha (*Dodonea viscosa*) and Lantana (*Tamarix aphylla* (Linn.) as oviposition attractant and larval growth promoter / inhibitor on House Fly, (*Musca domestica* L.). The plant chemicals in different final concentrations (25 and 50%) were mixed in a larval media prepared by mixing wheat bran, dried milk and brewer's yeast. Snatha acted as strong attractant for house fly with minimum larval duration (5.25 & 3.5 days), maximum larval (83.11 & 77.78 %) and pupal (73.22 & 71.11 %) survival, female sex ratio, intrinsic rate of increase (0.31 & 0.26), maximum biotic potential (0.67 & 0.77) and fecundity (259 & 242.4 egg female⁻¹). Lantana was a strong repellent for oviposition and showed a significant larvicidal activity. Gardenia showed maximum development time (11.20 days), less intrinsic rate of increase (0.213) and male sex ratio. Niazboo was repellent for oviposition (92.56 eggs) with minimum biotic potential (0.46), intrinsic rate of increase (0.19), less larval and pupal survival (55.56 & 54.00 %) at 50% concentration.

**EFFECT OF NEW FORMULATIONS OF NEEM PRODUCTS ON BIOLOGY OF
TRIBOLIUM CASTANEUM (HERBST) (TENEBRIONIDAE: COLEOPTERA)**

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The studies were conducted to evaluate the effect of neem based extracts on the eggs and larvae of *Tribolium castaneum*. Experiment on the efficacy of neem extracts against *T. castaneum* was conducted in the laboratory of Insect Toxicology, Department of Agri. Entomology, U. A. Faisalabad. Bioassay consisted of treated filter paper at the bottom of Petri dishes and covered with thin layer of wheat flour. Treatments comprised of 1.0%, 0.5 and 0.25% concentrations of each of neem products, viz., neem biopesticide, neem oil (cold press) and neemcos technical. The concentrations of each of the neem products had statistically non significant difference among them ($p < 0.05$) for larval and pupal and fecundity but had non significant difference in relation to larval and pupal durations. The reduction in fecundity as was observed in the present study reveals in limiting factor in population growth.

**PREDATOR FAUNA OF MAIZE, BAJIRA AND CHILLI PEST IN QASIMABAD,
HYDERABAD**RIFAQUAT ALI KAKA FARAZ ALI, MUKHTIAR ALI, TANVEER ALI, NASREEN
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Pakistan's economy depends upon the agriculture. Sindh is one of the important province that contributes a lot in the economy of Pakistan through agriculture. In Sindh many crops have been cultivated like Cotton, Maize, Barley, Sugar cane, Rice, Bajra, and many types of vegetables and fruits. Unfortunately these crops and vegetables are attacked by many pests due to the lack of attention to the pest management particularly the use of natural bio agents. Ghouri and Shah (1978) studied on the economic significance of maize pests and their control. As in nature the predators always be there where ever the pests. On account of this we have surveyed different crop fields of Qasimabad, Hyderabad. We started our survey from April to September 2009. We have visited fields weekly and observed the presence of predators in these fields, mostly the Beetles, such as *Coccinella undecimpunctata*, *Coccinella septempunctata*, *Chilomenes sexmaculata*, *Brumus sutaralis*, *Rodolia cordinalis*, Rove beetles. We have observed that *Coccinella septempunctata* was in greater number while *Brumus sutaralis* was in less number. We brought these beetles in the laboratory and observed their feeding behavior by providing them the Lepidopteron larvae of different species. We have also observed their feeding behavior in the field.

**THE URBAN COMMUNITY STRUCTURE OF BLOWFLIES OF HUAJIACHI,
HANGZHOU, EASTERN CHINA**

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The community structure of blowflies was determined in urban settings of Huajiachi, Hangzhou, eastern China. Standard liver-sodium sulfide baits were placed along various urban facilities, including open sewerage drain, garbage dump sites, mulberry plantation, college building, pig farm and meat-vegetable markets. (1) We collected 12 species in all with nine species of major forensic importance including *Chrysomya megacephala*, *Ch. pinguis*, *Aldrichina grahami*, *Lucilia sericata*, *L. illustris*, *L. porphyrina*, *Hemipyrellia ligurriens* (Diptera: Calliphoridae), *Musca domestica* (Muscidae) and *Parasarcophaga crassipalpis* (Sarcophagidae). (2) ANOVA test statistics revealed significant mean population variation between species ($F=251.670$) and months ($F=164.231$). (3) *Ch. megacephala* was the only species observed throughout the study year. This species was the most dominant and abundant group with a population mean of 33 ± 3.919 while *L. porphyrina* with a mean of 0.27 ± 0.122 was the least represented species. (4) A special assemblage of seven species excluding the blowfly *Ch. megacephala* and housefly *M. domestica* were found absent in the January of two consecutive years, i.e. 2007 and 2008. In February five species *L. sericata*, *L. porphyrina*, *L. illustris*, *H. ligurriens* and the flesh fly *P. crassipalpis* were absent in 2007-08 while in March only *L. porphyrina* and *H. ligurriens* were absent in 2007-08. Therefore, winter population means of all nine species were considerably low; January = 3.3 ± 0.246 , February = 1.14 ± 0.472 , December = 0.25 ± 0.76 and LSD analysis showed no significant difference for these months. However, statistical variation was significant when compared for winter and summer months.

SECTION - IV

PARASITOLOGY

PREVALENCE OF ZOONOTIC PARASITES IN DRINKING WATER OF NWFP

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Three different water sources (Tape water, ponds water and drain water) was easily available in three districts (Karak, Kohat and Hangu) were tested for zoonotic parasites. Identification of these parasites in water sources is important as these are increasingly recognized as causative agents of waterborne diseases. All the three sources of water were contaminated with eggs, cysts and oocysts. The results indicate a overall prevalence 65.5% (2955/450) contained protozoa, Amongst these were, *Giardia* 14.1% (63/450), *Cryptosporidium* 19.5 (88/450), *T. gondii* 2.8% (13/450), *F. hepatica* 4.8% (22/450), *E. coli* 5.78% (26/450) and *Entamoeba* 14.4% (65/ 450) respectively statistical analysis revealed $P < 0.05$. This study presents a need of an appropriate source of drinking water and identifying the threshold of water sources contamination that a health hazard which require treatment.

ON A NEW SPECIES OF NEMATODE PARASITE OF GENUS *RHABDOCHONA* RAILLIET, 1916 (NEMATODA: RHABDOCHONIDAE) FROM A PISCINE HOST, *CYPRINION WATSONI* (CYPRINIFORMES: CYPRINIDAE) OF BOLAN VALLEY, BALUCHISTAN

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A new species *Rhabdochona bifurcatum* n. sp. of rhabdochonid nematode is described parasitizing the intestine of the fish host *Cyprinion watsoni* (Day, 1872). The new nematode specimens differ in several diagnostic features compared with previously known species of the genus *Rhabdochona* Railliet, 1916. Unique to the new species is the presence of small spicule which is anteriorly bifurcated in to two unequal arms pointed at the tip. The present species is also characterized by having 8 prostomal teeth, 9 pairs of caudal papillae including 4 preanal and 5 postanal in males and smooth, non-filamented eggs in females. Excretory pore is postequatorial in both the sexes.

**REDESCRIPTION OF *PHYLLODISTOMUM RITAI* (KHAN, 1985) KHAN AND
BILQEES, 1990 FROM A NEW FRESHWATER FISH HOST
*HETEROPNEUSTES FOSSILIS***

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During a survey of trematodes of freshwater fish of Keenjhar Lake, Sindh, Pakistan. Three trematodes were recorded from a single host (*Heteropneustes fossilis*), these specimens were studied in detail and were found to be *Phyllodistomum ritai* (Khan, 1985) Khan and Bilqees, 1990 from a different fish host, earlier *P. ritai* has been recorded from the host *Rita rita*. This species is characterized in having body divided into two portions, the forebody narrow, while the hind body is flat, foliate and discoid. The cuticle is smooth. The body measures 6.16 to 6.19 by 3.58 - 3.66. The oral sucker is terminal and measures 0.64 - 0.66 by 0.48 - 0.49. The distance between oral sucker and caecal bifurcation 0.65 - 0.68. Prepharynx and pharynx are absent. The esophagus is elongated and measures 0.28 - 0.30 by 0.021 - 0.022. Caeca are thick and swollen. Acetabulum is 1.83 - 1.86 from anterior end of the body, almost round in shape 0.88 - 0.90 by 0.76 - 0.80 in size. Testes are intercaecal and lobed the right measuring 0.64 - 0.65 by 0.70 - 0.72 and the left 0.76 - 0.77 by 0.65 - 0.68. Distance between testis 0.44 - 0.45. Cirrus pouch is absent. Genital pore is post-bifurcal. Ovary is single, lobed at distance 0.36-0.38 from the ventral sucker measuring 0.40-0.42 by 0.40-0.41. Seminal vesicle elongated. No seminal receptacle. Vitellaria compact, post-acetabular paired at a distance of 0.047-0.049 from the ovary. Uterus occupying most of hind body. Excretory vesicle tubular. Eggs numerous, small, oval to rounded measuring 0.017-0.27 by 0.013-0.018.

**TWO NEW TREMATODES OF THE GENUS *ECTENURUS* LOOSS, 1907
(HEMIURIDAE: DINURINAE) FROM THE FISHES OF FAMILY
CARANGIDAE OF KARACHI COAST**

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KHAN

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Two new species of genus *Ectenurus* Looss, 1907 are described here. *E. karachiensis* n.sp, parasitizing *Caranx djedaba* and *E. malabaricus* n.sp in *Carangoides malabaricus*, *E. kasachiensis* is characterized by having elongate body, long soma and

relatively small tail, testes diagonal, postacetabular, seminal vesicle oval in shape, posterodorsal to acetabulum, pars prostatica is prominent. Hermaphroditic duct is short, sub cylindrical, genital atrium small, tubular, genital pore postbifurcal. Ovary is post-testicular, transversely elongate, vitellaria consisting of seven convoluted tubules, not reaching into tail, uterus extending into tail. Other species *E. malabaricus* has soma much broader than tail mainly at the junction with tail, testes are immediately postacetabular, flattened transversely, juxtaposed, seminal vesicle saccular, hermaphroditic duct is connected with pars prostatica which is posterior to it, genital atrium tubular, genital pore ventral at the base of pharynx. Ovary is close to testes, submedian; vitellaria composed of seven, thick, unequal tubules, not reaching into tail, uterus slightly extending into tail.

**AN ASSOCIATION OF PHYTONEMATODES ASSEMBLAGES ASSOCIATED
WITH POMEGRANATE IN KHUZDAR AND KALAT DISTRICTS,
BALOCHISTAN**

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A survey of phytonematodes associated with Pomegranate was conducted encompassing 12 localities of Balochistan province. In all twelve genera were recorded from the rhizosphere of Pomegranate. The most dominant species was *Meloidogyne incognita*. Species diversity (H') was highest in Wadh while lowest in Ali Dasht. Equitability (J') component of diversity was highest in Kork and lowest in Ali Dasht, while species richness (d') component was highest in Piromal. The soils associated with pomegranate orchards were coarse-structured, alkaline with low maximum water holding capacity.

***LECITHOCLADIUM CYBII* N.SP. (DIGENEA: HEMIURIDAE LUHE, 1901)
FROM THE FISH *CYBIUM GUTTATUM* OF KARACHI COAST**

NEELOFER SHAUKAT AND FATIMA MUJIB BILQUEES

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A new hemiurid trematode *Lecithocladium cybii* n.sp. is described here from the fish *Cybiium guttatum* (Cybidae) of Karachi coast. Present new species is separated from the previously described large number of species of the genus in having a combination of characters such as body long, soma long and wide at the level of ventral sucker, ecsoma

shorter and narrower ending into a knob posteriorly. Oral sucker terminal, transversely flattened, pharynx large, elongate, following into prominent oesophagus, the anterior part of which is distinct, sac-like, intestinal bifurcation is much anterior to ventral sucker, ventral sucker smaller than oral sucker, testes 2, close, diagonal, sub-globular in shape, situated almost in the middle of hind body, seminal vesicle elongate, bluntly pointed at both ends, anterior part of which is recurved followed by long, coiled pars prostatica, sinus sac extending from the posterior level of ventral sucker to mid region of pharynx and joining the long, tubular, genital atrium opening at the anterior margin of oral sucker. Ovary post-testicular, situated in posterior fourth quarter of soma, seminal receptacle present, vitellaria consist of seven, long, tubes, radiating ventral to seminal receptacle, all directed posteriorly. Uterine coils extend into middle of soma and anteriorly opening into the base of sinus sac. Eggs numerous. small. Excretory pore terminal.

***PLEORCHIS KARACHIENSIS* N.SP. (TREMATODA: PLEORCHIDAE, POCHE, 1926) FROM THE FISH *SCIAENA DUSSUMEIRI* OF KARACHI COAST**

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A new trematode of family Pleorchidae, Poche, 1926 *Pleorchis karachiensis* n.sp. is described here from the intestine of the fish *sciaena dussumeiri* Karachi coast. This species is characterized by having elongate body almost uniformly thick throughout its length with a rounded caudal appendage, oral sucker is terminal projecting out, rounded, prepharynx and esophagus prominent, pharynx transversely elongate, ceca long terminating anterior to caudal appendage, anterior cecal diverticula are absent, acetabulum small, spherical and a little behind cecal bifurcation, genital pore preacetabular, testes 50-52 in number, intercecal situated on each side of excretory bladder in two ventral and two dorsal longitudinal rows, cirrus sac claviform, extending laterally on right side of acetabulum and reaching in postacetabular region containing elongate seminal vesicle and pars prostatica. Ovary is median, 8 to 9 lobed, pretesticular, vitellaria consist of numerous small follicles extending from anterior level, post bifurcal to posterior end of the body proper but not reaching the caudal appendage, uterus coiled, between ovary and acetabulum, eggs numerous, elongate, metraterm prominent. Excretory vesicle tubular, excretory pore terminal.

A NEW TREMATODE OF GENUS *NEODICHADENA* YAMAGUTI, 1971 (DIGENEA: HEMIURIDAE LUHE, 1901: LECITHASTERINAE ODHNER, 1905) PARASITE OF THE FISH *TRIACANTHUS BREVIROSTRIS* (T.S) FROM FISH HARBOUR, KARACHI

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An undescribed species of hemiurid trematode of subfamily Lecithasterinae, *Neodichadena magnavesicula* n.sp. is reported and described here from the intestine of fish *Triacanthus brevirostris*. This is the first report of the genus *Neodichadena* from marine fish of Pakistan. The new species has an elongate body with subterminal, round, oral sucker, no prepharynx and esophagus, pharynx small, ceca long and wide. Acetabulum about three times larger than oral sucker. Testes two, small, postacetabular, seminal vesicle large, posterodorsal to acetabulum, pars prostatica tubular, hermaphroditic pouch near cecal bifurcation, genital pore at base of pharynx. Ovary is post-testicular, much wider than long, vitellaria 7 thick tubules, postovarian, seminal receptacle present. Uterus long, coiled, reaching to postvitelline zone and anteriorly joining the hermaphroditic pouch.

SEVEN NEW TREMATODES INCLUDING THREE NEW GENERA FROM FISHES OF KARACHI COAST

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Seven new digenetic trematodes including three new genera, *Macrorchirhynchus* n.gen. (Bucephalidae), *Neodiploproctodaetum* n.gen. (Leporeadiidae), and *Caudovitellaria* n.gen. (Hemiuridae). The species are *Macrorchirhynchus macrorchis* n.gen.; n.sp. from the fish *Psettodes erumei*; *Neodiploproctodaetum tetradontis* n.gen., n.sp. from the fish *Tetradon lunaris*; and *Caudovitellaria lobata* n.gen., n.sp. from the fish *Therapon jarbua*. Other four species are *Pseudodinosoma robustum* n.sp. (Hemiuridae) from the fish *Harpodon nehereus*; *Tubulovesicula microrchis* n.sp. (Hemiuridae) and *Tubulovesicula macrovesicula* n.sp. (Hemiuridae) from the fish *Plectorhynchus cinctus*; and *Bianium magnavesicula* n.sp. (Leporeadiidae) from the fish *Tetradon lunaris* of Karachi coast. *M. macrorchis* has very large testes near the anterior and close to rhynchus. *N. tetradontis* is peculiar in having wing-like lateral expansions, united at postacetabular region dividing the body into two regions. *C. lobata* is characterized by having vitellaria in two compact masses of 3 and 4 lobes, situated at

the posterior extremity of the body. *P. robustum* is with robust body, bipartite seminal vesicle and distal part of pars prostatica bulb-like *T. microrchis* is with very small testes and tubular seminal vesicle while *T. macrovesicular* has large seminal vesicle and elongate pars prostatica. *B. magnavesicula* has very large seminal vesicle occupying the whole intercecal field of body overlapping left ceca, anterior to testes.

**HISTOPATHOLOGICAL CHANGES ON SKIN OF *JOHNIUS MACULATUS*
(SCHNEIDER, 1801) INFECTED WITH PROTOZOAN PARASITE**

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Histopathological changes based on observations made on the skin of the fish *Johnius maculatus* infected with an unidentified protozoan are described. For this purpose histological sections were prepared by usual technique, stained with haematoxylin and eosin, dehydrated in graded series of alcohols, cleared in clove oil and xylene and mounted permanently in Canada balsam. Photographs were Prepared with Nikon (Optiphot-2) photomicroscope using a Fuji color film. Protozoan infection severely damaged the skin tissue, common findings were erosion, atrophy, dislocation of muscle fibers, shrinkage and degeneration of muscle fibre which produced large spaces inbetween the muscles fibers. Hyaline degeneration of muscle fibers was also prominent in some sections. Muscle fibers were severely damaged and epidermal and dermal layers were separated from each other.

**TREMATODES OF GENUS *TANAISIA* SKRJABIN, 1924 FROM KIDNEY OF
BLACK COOT *FULICA ATRA* (AVES: RALLIDAE) IN SINDH PROVINCE OF
PAKISTAN**

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During helminthological research on Black Coot *Fulica atra* (Aves: Rallidae) a total of 101 *Fulica atra* were collected from different water bodies in Sindh Province of Pakistan, The examination of birds revealed 47 trematodes of genus *Tanaisia* Skrjabin, 1924 from kidneys of six hosts. The species identified include; *Tanaisia manchhari* n. sp., *Tanaisia atra* (Nezlobinski, 1926), *Tanaisia fedtschenkoi* Skrjabin, 1924 and *Tanaisia longivittellata* Shtrom Skrjabin, 1947.

***MICROPHALLUS MAGNAGULLETUM* N. SP. (DIGENEA: MICROPHALLIDAE)
FROM INTESTINE OF BLACK COOT *FULICA ATRA* (AVES: RALLIDAE) OF
MANCHHAR LAKE, SINDH, PAKISTAN**

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During survey on helminth parasites of Black coot *Fulica atra* in Sindh Province of Pakistan, the trematodes belonging to the genus *Microphallus* Ward, 1901 were collected from intestine of the host bird. On the basis of body size, very broad esophagus, short irregularly lobed thick walled ceca, shape and position of testes and ovary, size of oral and ventral sucker and "V" shaped seminal vesicle, a new species *Microphallus magnagulletum* is proposed. The name of new species refers to broadest esophagus.

**FIELD EVALUATION OF TOMATO CULTIVAR/LINES FOR RESISTANCE
TO *MELOIDOGYNE INCOGNITA***

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The response of germplasms of tomato (*Lycopersicon esculentum* Mill.) was evaluated against *Meloidogyne incognita* infection in field environments. The purpose of this investigation was to find out resistant genotype (s) among seven commercially grown cultivars against root knot infection. The genotypes included in this study were Riogrande, Round-41, Round-27, Round small-127, PB-47, PB-28 and PB-8. Three week old seedlings were transplanted on ridges in a randomized complete block design with three replications. Transplants were spaced 30-cm apart in the row and 60-cm between rows. At 60 days, plants were uprooted, washed and ranked for root galling and egg mass indices on a 0 to 5 scales. The plant growth responses *i.e.*, foliage length, foliage weight, root length and root weight and nematode reproduction parameters *i.e.*, number of galls per root system, gall index, egg masses, egg mass index, eggs per root system, eggs per gram of root and second stage juveniles per 100-cm³ of soil were also recorded. The field experiment revealed that *M. incognita* was able to infect, cause root galling and reproduced on all the seven tomato genotypes. All the cultivars were susceptible to *M. incognita* infection but response was variable. Tomato cv. PB-8 and PB-28 were susceptible with gall index of 4. While other five cultivars namely Round-41, Riogranade, Round-27, Round small-127, and PB-47 were highly susceptible having 105 to 133 galls per root system and gall indices of 5. The findings of this trial proved that there is no resistant cultivar for grower to recommend. We will suggest that the field with high nematode population should be rotated with non host crops such as cereals.

**TEESATODIPLOSTOMUM MEGABURSUM GEN. N., SP.N. (TREMATODA:
DIPLOSTOMIDAE POIRIER, 1886) FROM THE BIRD WHITE-EYED
BUZZARD BUTASTUR TEESA (ACCIPITRIDAE)**

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A new genus *Teesatolip/ostomum* is described from the intestine of the bird white-eyed buzzard *Butastur teesa* (Accipitridae). The new genus is characterized by having small, bipartite body, forebody oval, and hindbody cylindrical. Tegument is smooth. Oral sucker terminal, smaller than ventral sucker. Pseudosuckers absent. Ventral sucker round, larger than oral sucker, anterior to tribocytic organ. Tribocytic organ massive, oval, with median silt, located posterior to ventral sucker. Intestinal ceca terminating near posterior extremity. Testes two, tandem, submedian, smooth, oval, separated, anterior testis in anterior half of hindbody and posterior testis in posterior half of the hind body. Bursa voluminous. Ovary intertesticular, submedian, and ovoid. Vitellaria extending from mid level of acetabulum to posterior extremity, consisting of numerous dense and opaque follicles. Eggs are large.

**SINDHUDIPLOSTOMUM ELONGATUM GEN. N., SP. N. (TREMATODA:
DIPLOSTOMIDAE) FROM COMMON EURASIAN KINGFISHER, *ALCEDO
ATTHIS* (ALCEDINIDAE)**

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A new genus *Sindhudip/ostomum* is described from the intestine of Common Eurasian Kingfisher *Alcedo Atthis* collected from Matiari, Hyderabad, Sindh, Pakistan. The new genus is characterized by having, bipartite body. Tribocytic organ large, almost round, hindbody long and cylindrical. Oral sucker present, ceca terminating near posterior extremity. Testes tandem, in hindbody and there is no cirrus pouch, genital atrium present, genital pore dorsosubterminal. Ovary intertesticular. Vitellaria follicular, only in hind body. The species name *Sindhudip/ostomum elongatum* is proposed due to relatively elongated hind body.

**ACANTHOCEPHALA FROM A BIRD (*ACCIPITER BADIUS* (SEVERTZOV)
FROM KARACHI, SINDH, PAKISTAN**

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During a survey of acanthocephalan parasites of birds, eight specimens were recorded from small intestine of a single bird host (*Accipiter badius* Severtzov). On detail study it was found that the specimens belonged to the genus *Mediorhynchus* Van Cleave, 1916. The specimens possessed the following characters: Body medium sized without pseudosegmentations. Proboscis with hooks in spiral rows in the anterior portion and small spines on the posterior portion, posterior portion wider than the former portion. Proboscis receptacle single walled and saccate. Lemnisci long and slender. Testes two, oval to elongate. Cement glands oval and prominent. Eggs oval with concentric membranous shells. The specimens are being compared with the known species of *Mediorhynchus* recorded from Indo-Pakistan and other parts of the world.

**HOST SEARCHING ABILITY AND PARASITIZING POTENTIAL OF
TRICHOGRAMMA CHILONIS (ISHII) (HYMENOPTERA:
TRICHOGRAMMATIDAE) IN CAULIFLOWER**

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Field experiments were conducted at NIAB, Faisalabad, during year 2008-2009 in cauliflower field to find out host searching ability and parasitizing potential of an egg parasitoid, *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) at distances from 1 to 21 metre from parasitoids release point. All standard agronomic practices were used throughout the growth of the vegetable under pesticide free conditions. Eggs of factitious host, *Sitotroga cerealella* were pasted on paper card and tagged on leaves of cauliflower plants at distance all around in the field after 24, 48 and 72 hours release intervals to check the parasitism. Results showed that parasitoid's searching for host was very successful and eggs were parasitized at all distances. Parasitism varied with time intervals and it was 66-70, 86-90 and 35-46% after 24, 48 and 72 hours respectively. Results conclude that *T. chilonis* can travel in field to a distance of 21 metre with a significantly high percent parasitism.

NEMATODES ASSOCIATED WITH SIXTEEN FLOWERING PLANTS

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This investigation was conducted to identify the nematodes associated with flowering plants, their distribution, and population dynamic. Sixteen summer and winter flowering plants including Petunia (*Petunia hybrida*, Family: Acanthaceae), Pansy (*Viola wittrockiana*, Family: Violaceae), Mary's Gold (*Warszewiczia coccinea*, Family: Rosaceae), Prime Rose (*Hibiscus syriacus*, Family: Phytolaccaceae), Paper flower (*Betula papyrifera*, Family: Helenieae), Phlox (*Phlox*, Family: Polemoniaceae), Cocks Comb (*Erythrina crista-galli*, Family: Amaranthaceae), Cornflower (*Centaurea cyanus*, Family: Compositae), Hollyhock (*Althea rosea*, Family: Malvaceae), Calendula (*Calendula officinalis*, Family: Compositae), Larkspur (*Delphinium parishii*, Family: Ranunculaceae), Snapdragon (*Collinsia parviflora*, Family: Figwort), Lavender (*Lavandula officinalis*, Family: Labiatae), Dianthus (*Dianthus armeria*, Family: Caryophyllaceae), Ornamental Cabbage (*Brassica oleracea*, Family: Brassicaceae) and Sweet Pea (*Lathyrus odoratus*, Family: Fabaceae). The root and soil samples were collected from various nurseries located in Faisalabad city. Three cores consisting of roots and rhizosphere soil for each flowering plant were collected with Oak Land tube. The root and soil samples were processed separately. The nematodes were extracted from a root composite sub-sample of 20 g by placing in a mist-chamber for 5 days. A sub-sample of 100 cm³ of soil was screened through nested 100 and 320 meshes. The filtrate was loaded on Baermann funnel and placed in a mist-chamber for 3 days. Root galling was assessed on 0 to 5 scales. Sixteen ornamental flowers belonging to fifteen families were found infected with four genera of plant parasitic nematodes namely *Meloidogyne*, *Pratylenchus*, *Hoplolaimu* and *Ditylenchus*. Root knot nematode *M. incognita* and *P. vulnus* are the damaging pathogens of roses and many other. The root galling index ranged from 1 to 3.5.

NEMATODES ASSOCIATED WITH FRUIT TREES

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This research was conducted to find out the presence of plant parasitic nematode with fruit trees planted at the campus of University of Agriculture Faisalabad. Plant parasitic nematodes belonging to ten genera including *Criconemella*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Radopholus*, *Rotylenchulus*, *Trichodorus*, *Tylenchorhynchus*, *Tylenchulus*, and *Xiphinema* in roots and soil of 22 Plant species were identified. Host of

these nematodes were *Apricot* (*Prunus Armeniaca*, Family: Rosaceae), *Avocado* (*Persea Americana*, Family: Lauraceae); *Blackberry* (*Robus argatus*, Family: Rosaceae); *Banana* (*Musa* spp, Family: Musaceae); *Blueberry* (*Vaccinium corymbosum*, Family: Ericaceae); *Fig* (*Ficus carica*, Family: Moraceae); *Grape* (*Vitis* spp, Family :Vitaceae); *Guava* (*Psidium guajava*, Family: Myrtaceae); *Jujube* (*Ziziphus jujub*, Family: Rhamnaceae); *June Plum* (*Spondias dulci*, Family: Anacardaceae); *Loquat* (*Eriobotrya japonica*, Family: Rosaceae); *Lychee* (*Litchi chinensis*, Family: Sapindaceae); *Mango* (*Mangifera indic*, Family: Anacardiaceae); *Mulberry*, *Black Mulberry* (*Morus nigr*, Family: Moraceae); *Olive* (*Olea europaea*, Family: Oleaceae); *Papaya* (*Carica papaya*, Family: Caricaceae); *Peach* (*Prunus persica*, Family: Rosaceae); *Persimmon* (*Diospyros kaki*, Family: Ebenaceae); *Pineapple* (*Ananas comosus*, Family: Bromeliaceae); *Raspberry* (*Robus niveus* ,Family: Rosaceae); *Orange* (*Citrus medica*, Family: Rutaceae); and *Pomegranate* (*Punica granatum*, Family: Punicaceae). Most destructive nematodes might be root knot nematode, *Meloidogyne* spp, root lesion, *Pratylenchus* spp. and reniform nematode *Rotylenchulus* spp. due to their high population with roots of host plants.

RESPONSE OF EGGPLANT (*SOLANUM MELONGENA*) CULTIVARS TO *MELOIDOGYNE INCOGNITA* INFECTION

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An experiment was conducted to screen ten eggplant cultivars against *Meloidogyne incognita* infection in the green house at 25±4°C. Three week old seedlings of egg plant cultivars including Dilnasheen, VRIB-04, VRIB-01, VRIBH-03, Clusterbrking, Faisalabad-Long, PPR, Purple Queen, Nirala and Bamissal were transplanted into 15-cm clay pots filled with sterilized soil. There were ten treatments with five replications for each. Seven days after planting, the plants were inoculated with freshly hatched 1000 J2 of *M. incognita*. The plants were allowed to grow for 60 days. At harvesting, plants were uprooted and washed carefully under tap water to remove the adhering soil. The roots were stained with Phloxine B to assess the egg masses. The root galling and egg masses indices were assessed on 0 to 5 scale, Where 0 = no galls/egg masses, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 5 = >100 galls or egg masses per root system. Presence of galls and egg masses on the root of all the eggplant cultivars revealed that none of the eggplant germplasm was immune to root knot nematode infection. The root galls among ten cultivars were variable in number and size. The size of galls on roots of Nirala cultivar was smaller about the size of pinhead whereas on root of four cultivars including VRIB-01, VRIB-04, VRIB-03 and Faisalabad Long was intermediate and on the root of all other 5 cultivars namely Purple Queen, Bimissal, Clusterbrking, PPR and Dillnasheen was large about the size of pea. The roots of Nirala contain significantly ($P=0.05$) less number of galls (45) and egg masses (51) compared to that of all other nine

cultivars. Nine cultivars including Dillnasheen, Purple Queen, VRIB-01, Faisalabad long, PPR, Bamissal, VRIBH-03, VRIB-04 and Clusterbrking had 5 root galling and egg masses indices, whereas Nirala had 4. These findings had demonstrated that egg plant is a good host of *M. incognita* so the growers must rotate the infected field with poor host crops like cereals to keep the nematode population below threshold.

NEMATODES ASSOCIATED WITH ORNAMENTAL SHRUBS

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This study was conducted to assess the association of pant parasitic nematodes and their population level with 19-ornamental shrubs. The shrubs sampled included Aloe Vera (*Aloe vera*, Family: Liliaceae); Ashoka (*Saraca indica*, Family: Caesalpiniaceae); Blazing rose (*Iresine herbstii*, Family: Amaranthaceae) Bougainvillea (*Bougainvillea glabra*, Family: Nyctaginaceae); *Brazillian red hot* (*Alternanthera dentate*, Family: Amaranthaceae) Chadna (*Abelmoschus esculentus*, Family: Malvaceae); Chadni (*Gypsophila elegans*, Family: Caryophyllaceae); Din ka raja (*Cestrum diurnum*, Family: Solanaceae); Duranta (*Duranta erecta*, Family: Verbenaceae); Ficus (*Ficus religiosa*, Family: Moraceae); Gardenia (*Gardenia jasminoides*, Family: Rubiaceae); Golden duranta (*Duranta repens goldii*, Family: verbenaceae); Gul-e-fanoo (*Kigelia pinnata*, Family: Bignoniaceae); Hemilia (*Abelmoschus esculentus*, Family: Malvaceae); Money plant(*Crassula ovata*, Family: Crassulaceae); Murva (*Chonemorpha macrophylla*, Family: Apocynaceae); Rat-ki-rani (*Cestrum nocturnum*, Family: Solanaceae); Shoe flower (*Hibiscus* spp. Family: Malvaceae); and Table palm (*Ferula asafoetida*, Family: Apiaceae). The root and soil samples were collected from the shrubs planted on the campus of University of Agriculture, Faisalabad. Each sample was of three core from each shrub. The root and soil samples were processed separately. The nematodes were extracted from a root composite sub-sample of 20-g by placing in a mist-chamber for 5-days. A sub-sample of 100 cm³ of soil was loaded on Whitehead and Hamming tray to extract J2 for 3-days. Plant parasitic nematodes belonging to seven genera including *Belonolaimus*, *Circonemella*, *Helicotylenchus*, *Meloidogyne*, *Paratylenchus*, *Rotylenchulus* and *Xiphinema* were identified for nineteen plant genotypes belonging to fifteen different families. The population of nematodes varied from plant to plant. Tow species of root knot nematode (*M. incognita* and *M. javnica*), and one of ring nematode, *Circonemella xenoplax* are considered to be destructive nematodes of shrubs. The level of nematode population varied from sampling site to site. Root galling and egg mass indices by root knot nematode ranged from 2 to 5 on a scale of 0 to 5.

MANAGEMENT OF ROOT KNOT NEMATODE *MELOIDOGYNE INCOGNITA* BY PLANT GROWTH PROMOTING RHIZOBACTERIA ON TOMATO

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This study was planned to assess the efficacy of plant growth promoting rhizobacteria (PGPR) against *Meloidogyne incognita* infection on roots of tomato (*Lycopersicon esculentum* Mill.) in the green house at 30±4°C. Fifteen-days-old seedlings of tomato cultivar "Money Maker" were planted singly in 15-cm-diam pots filled with sterilized sandy soil. Two days after transplanting, 20-ml of 5% sugar solution containing 10⁷ CFU/ml each of *Bacillus* spp., *Azotobacter* spp., *Pseudomonas putida* and *P. fluorescens*, were pipetted into three 3-cm deep holes surrounding the root zone of each plant. Five days after the application of PGPR, freshly hatched 2000 J₂ were applied at root zone. The experiment consisted of seven treatments; each with three replicates and arranged in CRD. Pots with nematodes, without nematodes, and PGPR were kept as control for comparison. Sixty days after inoculations, data of plant growth parameters such as plant height, fresh and dry root and shoot weight and nematode reproduction in term of egg masses and galling index per root system were recorded. The plants treated with *P. fluorescens* significantly ($P = 0.05$) suppressed egg masses (41.50%) and galling index (32.05%) resulting in improved growth over control plants. The treatments having *P. putida*, *Bacillus* spp. and combination of PGPR showed intermediary effects on both nematode reproduction and plant growth. *Azobacter* spp. was least effective in suppressing only 29.38% 20.04% egg masses and galling index, respectively with no effect on plant growth.

HYPERSENSITIVE REACTION AND CROSS-PROTECTION IN GRAPE AGAINST AGGRESSIVE ROOT KNOT NEMATODE *MLOIDOGYNE ARENARIA*.

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Pre-inoculation of the host with an avirulent pathogen can induce cross protection against subsequent infection by virulent pathogen; this is called "induced resistance". *Meloidogyne arenaria*, avirulent population and *M. incognita*, an avirulent population demonstrated host specificity on grape rootstocks. One-half of a split root system of

plants of Harmony grape rootstock resistant to *M. incognita* but not to *M. arenaria* were pre-inoculated with an avirulent population of *M. incognita* to induce host resistance against virulent *M. arenaria* population. Zero, 7, 13, 21, and 27 days later, the other half was challenged with virulent *M. arenaria*. Host suitability was determined on the bases of reproduction factor (Pf/Pi , where Pf = final nematode population / Pi = initial population) 63 days after being challenged by virulent nematode population. Prior infection with avirulent *M. incognita* significantly suppressed reproduction of challenge *M. arenaria* applied 5 days after or subsequently. Rate of reproduction of virulent *M. arenaria* on adjacent roots of Harmony was 72, 45, 12, 7 and 6 when determined at 0, 7, 13, 21, and 27 days inoculation intervals, respectively. The level of protection increased with increased level of avirulent nematode. The rate of reproduction of virulent nematode population was 73, 66, 54, 18 and 14 at 0, 500, 1,500, 5,000 and 10,000 eggs per half root system, respectively. These findings revealed that prior infection of plants with avirulent nematode make the plant resistant to subsequent nematode infection.

COMPARITIVE STUDY OF VARIOUS CONCENTRATIONS OF RINGER'S LOCK SOLUTION ON THE SURVIVAL OF GASTROINTESTINAL NEMATODES IN SMALL RUMINANTS

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Sheep and goat farming is an important activity of our country particularly in arid areas, as they adopt well in adverse condition too. Infestation of sheep and goat with gastrointestinal nematodes result in loss of their productivity and thus affect the farmer's income. So these gastrointestinal nematodes are the much focused issue of veterinary scientists. Among these issues the important one is the survival of mature worms out side the host as for scientific activity their survival is must. For this purpose current study was designed with the aim to test the Ringer Lock solution as a survival medium. For this purpose, four different concentrations *i.e.* 100, 75, 50 and 25 per cent respectively were prepared. Same percentages of Phosphate Buffer Saline solution were also were also prepared and taken as control. Gastrointestinal nematodes were collected from freshly slaughtered sheep and goat and immediately transferred to solution bottle kept in temperature control box and they were transferred to the temperature controlled (37° C) Petri plates filled with 10 ml of respective solution. The worm survival as then mentioned for the period of 120 hours. The result revealed that among these solutions, a Ringer Lock concentration of 75 percent showed higher worm survivability compared to all other solutions including controls as 50% worm survived in this solution till 72 hours. So we concluded that Ringers Lock at the concentration of 75 percent showed higher nematodes survivability. Further broad based and multidimensional studies are needed in this respect.

THE INCIDENCE OF INTESTINAL PARASITES IN BUFFALOES IN FATEH JANG

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Gastrointestinal parasites infection in buffaloes is one of the major problems in buffalo production. Moreover, these parasites are responsible for loss in body weight, decrease feed efficiency, reduce growth rate, delay maturity, poor body conditions, anemia and low resistance to various other diseases. This study was designed to find out incidence of various GI parasites in buffaloes in area of Fateh Jang. Fecal samples of 74 buffaloes of both the sexes from the age of one month to more than 36 months were collected directly through rectum in plastic bags. Eggs/larvae were identified according to standard key. Out of 76 samples, 42 (55.26 percent) were found infected with various parasites. Infection rate was found higher in female buffaloes (68.42 percent) as compare to male buffaloes (31.57 percent). Incidence of infection is greater at very young ages (1-3 months: 5.22 percent) and older ages (>36 months: 16.22%). Total 9 species of parasites were observed in fecal samples of buffaloes. *Neoscaris vitulorum* had found in highest frequency in both male and female buffaloes *i.e.* 65 (23.30 percent) and 123 (24.12 percent) respectively.

ECHINOCHASMUS INDUSI NEW SPECIES (TREMATODA: ECHINOSTOMATIDAE) FROM POND HERON ARDEOLA GRAYII (AVES: ARDEIDAE) OF JAMSHORO, SINDH, PAKISTAN

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A new trematode *Echinochasmus indusi* is described from intestine of Pond Heron *Ardeola grayii* (Aves: Ardeidae) collected from Jamshoro, Sindh, Pakistan. The present worm resembles with *E. zubedakhaname*, *E. leopoldinae* and *E. donaldsoni* in having 20 collar spines; whereas, it differs from *E. zubedakhaname* and *E. leopoldinae* in body length, arrangement of corner cephalic spines, shape of posterior body, head collar, testes, smaller vitelline follicles and large pharynx, acetabulum and eggs. It also differs from *E. donaldsoni* in having smaller body size, oral sucker, esophagus and large acetabulum, pharynx, and egg size. Due to aforementioned differences the present specimen is proposed as *Echinochasmus indusi*, the name of species refers to River Indus.

***ECHINOCHASMUS ARDEOLAE* NEW SPECIES (TREMATODA:
ECHINOSTOMATIDAE) FROM POND HERON *ARDEOLA GRAYII* (AVES:
ARDEIDAE) OF HYDERABAD, SINDH, PAKISTAN**

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During the survey of helminth parasites of bird Pond Heron *Ardeola grayii* (Aves: Ardeidae) in Hyderabad and Jamshoro Districts of Sindh Province Pakistan. A new trematode *Echinochasmus ardeolae* is described from gut contents of host. The present specimen closely resembles to *E. accipiteri* in most of the features, but differs in arrangement, shape and number of collar spines, body length and distance of acetabulum from anterior extremity, median position of ovary, rounded pharynx as well as different avian host. On the basis of differentiating diagnostic features, a new species *Echinochasmus ardeolae* is proposed. Name of the new species refers to the generic name of the host.

***PELMATOSTOMUM MUJIBI* SP. N. (TREMATODA: ECHINOSTOMATIDAE)
FROM THE LARGE EGRET *EGRETTA ALBA* IN KARACHI, SINDH,
PAKISTAN**

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Two specimens were recovered from the small intestine of a common large egret *Egretta alba*. The genus *Pelmatostomum* Dietz, 1909. in Karachi, sindh. These were fixed, dehydrated, stained and mounted permanently. Detailed study, revealed that these belonging to a new species and are characterized by having a: small, elongate and slender body. Head collar biscuit-shaped, with dorsally uninterrupted row of 30 spines. Oral sucker small. Prepharynx Moderate in length. Oesophagus is fairly long. Pharynx elongated and oval in shape. Acetabulum is larger than the oral sucker, near anterior extremity. Ovary median, immediately in front of anterior testis. Testes are oval to elongated, directly tandem at posterior extremity. Cirrus pouch apparently absent; ejaculatory duct very long, seminal vesicle with muscular wall, reaching far back of acetabulum. Uterus long, with numerous windings; eggs numerous, with large operculum and distinct eye spots. Vitellaria is confined to postequatorial lateral fields and do not extend back of anterior end of anterior testis.

LUBENS SINDHENSIS SP.N. (TREMATODA: DICROCOELIDAE) FROM THE SMALL INTESTINE OF *PHYLLOSCOPUS TYTIERI* (BROOKS) IN KARACHI, SINDH

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Two out of four birds *Phyllscopus tytleri* (Brooks) harboured nine trematodes in the small intestine. These were fixed, dehydrated, stained and mounted permanently. A detailed study revealed that these belong to a new species, designated as *Lubens sindhensis* sp. n. The new species is characterized by having an oval, elliptical body with posterior extremities either pointed, or with a sharp lateral cut or having a much broader posterior end, in having a ventral sucker larger than the oral sucker, in having ventral sucker 2.1-2.5 away from cecal bifurcation and in having oval rounded testes and ovary with a small seminal receptacle. *Phylloscopus tytleri* is a new host record.

NEPHROSTOMUM SP. DIETZ, 1909, FROM THE LARGE EGRET *EGRETTA ALBA* (Syn. *ARDEA ALBA*) IN KARACHI, SINDH, PAKISTAN

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Two large Egrets *Egretta alba* (Syn. *Aldea alba*) were caught from the suburbs of Karachi city. The birds were anesthetized, autopsied and examined for helminth parasitic infections; A single trematode was recovered from small intestine of the bird. The specimen was fixed, dehydrated, stained and mounted permanently according to the standard procedures. The specimen was studied and identified as belonging to the genus *Nephrostomum* Dietz, 1909. The trematode is characterized by having an elongate, medium-size body, reniform head collar, with shallow dorsal incision, and single, dorsally uninterrupted row of 34 spines, which are very small dorsally and become larger towards the sides. Oral sucker is sub terminal oval to elongate in shape. Acetabulum is produced backward, in first quarter of the body. Ovary is ovoid, pearl-shaped, median, equatorial. Uterus long profuse with several eggs, strongly winding. Testes are elongated, irregular in shape, indented, median, post equatorial, Cirrus pouch small, over reaching acetabulum. Eggs are numerous. Vitellaria lateral, extending from posterior extremity to level of posterior end of acetabulum.

**A RECORD OF THE GENUS *LYPEROSOMUM* SP. LOOSS, 1889
(TREMATODA; FAMILY DICROCOELIIDAE) FROM THE *EGRETTA ALBA*
SYN. *ARDEA ALBA* IN KARACHI, SINDH, PAKISTAN**

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A single specimen belonging to the genus *Lyprosomum* Looss, 1889 was recovered from the alimentary canal of the bird *Egretta alba* Syn. *Ardea alba* in Karachi, Sindh. It was fixed, processed, stained and mounted permanently according to the standard procedures. A detailed study revealed that the body is delicate, elongated, and slender. Oral sucker is sub terminal, well developed and rounded in shape. Oesophagus is short. Ceca terminate some distance short of posterior extremity. Acetabulum is sLib equal to oral sucker; it is oval and rounded in shape, situated with in anterior third of body. Ovary is irregular in shape, post testicular, in middle third of body, smaller than the testes. Testes are larger than the ovary, quite irregular in shape, tandem, postacetabular separated through the uterine coils. Cirrus pouch anterior in position, pre-acetabular. Genital pore at level of posterior end of pharynx. Uterine coils occupy most of the hind body. Eggs are small, brown and thin shelled. Vitellaria commence at the level of ovary up to some distance behind it. Excretory vesicle tubular.

**SECTIONS OF SPLEEN OF RED SNAPPER, *LUTJANUS*
ARGENTIMACULATUS (FORSK., 1775) INFECTED WITH *ANISAKIS* SP. AND
CONTRACAECUM SP. LARVAE (NEMATODA:ANISAKIDAE &
HETEROCHEILIDAE)**

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The present investigation deals with the pathological changes in the spleen of marine fish *Lutjanus argentimaculatus* (Forsk.,1775) infected with nematode larvae. Nematode larvae of the genus *Anisakis* sp. and *Contracaecum* sp. were found attached and embedded in the spleen of fish. For the study of the changes in the spleen of fish due to the infestation of nematode larvae, histopathological sections were prepared and stained by routine procedure. Photographs of selected portions were prepared in support of damages caused by the nematode larvae. Gross anatomy of the attachment site shows numerous small pores and damages in the spleen. It appeared ruptured with parts of the outer surface eroded. In histological sections large numbers of melanomacrophage. centers were observed. It was also observed that blood vessels undergo degenerative changes and fibrosis of vessel wall was obvious resulting into thickness of the wall. Sinusoids also appeared dilated and degenerated.

**FIRST RECORD OF *NEODIPLOSTOMUM ORCHILONGUM*, NOBLE, 1936.
(TREMATODA: DIPLOSTOMATIDAE) FROM PAKISTAN IN BIRD POND
HERON *ARDEOLA GRAYII* (AVES: ARDEIDAE) OF JAMSHORO, SINDH,
PAKISTAN.**

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A single worm collected from intestine of Pond Heron *Ardeola grayii* (Aves: Ardeidae) in Jamshoro, Sindh, Pakistan is described. The fluke under present study resembles *Neodiplostomum orchilongum*, Noble, 1936; in all essential features; therefore, identified as such. However, the present species is being reported for the first time from Pakistan and also a new host record.

**STUDIES ON ACANTHOCEPHALA FROM THE BIRDS OF PAKISTAN WITH
DAMAGES CAUSED BY THEM IN THE INTESTINE OF *PASSER
DOMESTICUS* (LINNAEUS, 1758)**

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The acanthocephalan parasite constitute an important group of worms, unlike trematodes they lack a mouth and digestive system, usually the life cycle of these worms are simple as compared to trematodes. However most acanthocephalans require atleast one or two intermediate hosts. In Pakistan, parasitology on the whole is a neglected field of study, inspite of the fact that parasite infections are one of the major cause of disease in human and animals. In Pakistan researchers and students always face difficulty in locating the parasite fauna of this region specially the birds, therefore it is thought necessary to prepare a list of acanthocephalan found in birds of this region, because many species of acanthocephalan found in Pakistan are not reported yet. During the present study, some species of acanthocephalan were also recovered which were presented here. Permanent slides were prepared by standard techniques, after fixing the parasites in FAA solution. Observations and results were prepared in the form of drawings. Intestinal histopathology related to acanthocephalan infection has not been studied previously except eagle. Therefore the present studies are undertaken to describe the damage caused by the recently described species *Mediorhynchus papilosum* (Van Cleave, 1916) in the intestine of *Passer domesticus*. For this purpose portions of infected intestine were processed for microtomy by standard procedure and H & E stained were used for staining

the tissues and permanently mounted sections were prepared. Observations revealed that serious damage is caused in the intestine of sparrow by *Mediorhynchus papilloslim* (Van Cleave, 1916). This include necrosis and atrophy of villi in particular and of the intestinal wall in general. Total destruction of villi was seen at the site of infection even the sites where worm was not physically present specially the surface of mucosal region. The necrotic areas in the vicinity were infiltrated with inflammatory cells including few neutrophils, plasma cells and lymphocytes. Hemorrhagic necrosis was also obvious in certain parts of the intestine. It is concluded that severe tissue damage occurred in the intestinal wall of sparrow due to this acanthocephalan infection. Especially villi are badly damaged loosing their usual upright position, being necrotic and atrophied. Extensive mucous exudates also observed in the damaged portion of intestine. Photographs of selected sections were taken in support of observations.

**A NEW SPECIES OF THE GENUS *CENTRORHYNCHUS* LUHE, 1911
(ACANTHOCEPHALA: GIGANTORHYNCHIDEA: CENTRORHYNCHIDAE)
FROM THE SNAKE *VIPERA RUSSELLI* (SHAW, 1797) OF KARACHI, SINDH
(PAKISTAN)**

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A new acanthocephalan *Centrorhynchus karachiensis* n. sp. Is described here collected from the intestine of *Vipera russelli*. Specimens were fixed in AF A solution, stained with alum carmine and mounted permanently by, usual methods. The new species is characterized by having short body, long neck and long spines on proboscis. Proboscis is cylindrical and small bearing 14- 16 longitudinal rows of spines. Proboscis receptacle double walled, in male leminisci longer than proboscis receptacle. Eggs are shorter as compared to the previously described species. Reproductive system is well developed. Testes are two and small. Bursa well developed and saeftigen's pouch is small. Key Words: Snake, *Vipera russelli*, Acanthocephala, *Cenirorhynchus* sp., Karachi, Sindh.

**IMMUNOHISTOCHEMISTRY OF KIDNEY OF *BUBALUS* SP. INFECTED BY
*FASCIOLA HEPATICA***

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Fascioliasis is an important parasitic disease that effects livestock and man worldwide and cause significant economic losses and great expense with antihelmintics. In Pakistan also it lowers production of milk, meat and wool, reduced weight gain and

production loss due to mortality. During the period of study, 26 animals of *Bubalus* sp. were examined for fascioliasis and kidney specimens were collected. 14 animals of *Bubalus* sp. were parasitized by *Fasciola hepatica* while kidney of 11 animals were found uninfected and further studies carried out by utilizing microtome procedure using H & E stains which revealed the glomerulonephritis. Some infected sections were also studied for immunohistochemistry which is done by standard technique by using Masson's Trichrome. Sections of immunohistochemistry revealed membranoproliferative pattern and psudolinear IgG deposits. So our study concluded that glomerulopathy has been showed in kidney of buffaloes by *Fasciola hepatica* infection and it leads to severe clinical manifestations including hematuria, mild proteinuria, nephritic syndrome and chronic renal failure. This effects the growth, reproduction and milk production of the host.

**IN VITRO VALIDATION OF ANTHELMINTIC ACTIVITY OF SOME
BOTANICALS USED IN TRADITIONAL VETERINARY MEDICINE IN
PAKISTAN**

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Development of resistance in parasites to several families of drenches and chemical residues and toxicity problems has posed a threat in effective chemotherapeutic parasite control programme. These considerations have revived interest in exploiting the potential of medicinal plants for their use as anthelmintics. This paper validates use of *Melia azedarach* (bark), *Fumaria parviflora* (whole plant), *Syzgium cuminii* (bark), *Mallotus philippinensis* (fruit), *Caesalpinia bonduc* (seeds), *Eremostachys vicaryi* (seeds) and *Vernonia anthelmintica* (seeds) as anthelmintics in the traditional veterinary medicine in Pakistan. *In vitro* evaluation for anthelmintic activity of crude methanol extracts (CME) of the plants was carried out using egg hatch test (EHT), larval development assay (LDA) and adult motility assay (AMA) using *Haemonchus contortus* eggs and worms. *M. azedarach* and *F. parviflora* exhibited potent ovicidal effects with LC₅₀ values of 407.38 and 416.87 µg/mL, respectively; whereas, *S. cuminii*, *M. philippinensis*, *C. bonduc*, *E. vicaryi* and *V. anthelmintica* were effective *H. contortus* as all the worms were found dead from 6-12 hours post-exposure to CME at 4000-8000 µg/mL. In LDA, anthelmintic activity of plants included in the study was not significant. All the plants exhibited dose-dependent effects. The plants validated for their anthelmintic activity may be subjected to further scientific studies for drug development.

**HISTOPATHOLOGICAL CHANGES IN THE INTESTINE OF
PLECTORHYNCHUS CINCTUS (TEM&SCHL.) ASSOCIATED WITH
NEMATODE LARVAE**

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Plectorhynchus cinctus (Tem&Schl.) is a popular edible fish of Karachi coast, belonging to family Pomadacyidae commonly called as Gisser. The nematode larvae are identified belonging to genus *Anisakis*. Histopathological sections of intestine of the infected fish were prepared by standard procedure, stained with hematoxylin and eosin and mounted permanently in Canada balsam by usual techniques. For this purpose fishes were collected from West Wharf Karachi. Microphotographs of selected sections were prepared and observed. The observation revealed that intestinal villi were totally destroyed and tunnel formation was seen clearly. Intestinal mucosa was badly affected, hemorrhage, inflammation, adenoma, ulceration, atrophy, necrosis, degeneration and erosion of the surface layers was seen in several sections. A condition like atrophic muscularis mucosae was also common. Submucosa was swollen and most of it was replaced by homogeneous pink staining material and fibrinous exudates.

**FIRST REPORT OF GENUS *CENTRORHYNCHUS* LUHE, 1911
(ACANTHOCEPHALA: CENTRORHYNCHIDAE) IN *STURNUS VULGARIS*
WITH DESCRIPTION OF A NEW SPECIES IN SINDH, PAKISTAN**

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A total of 06 *Sturnus vulgaris* were examined for the presence of helminth parasites. The examination of gut contents and visceral organs revealed 07 acanthocephalans (06 males and 01 female) belonging to the genus *Centrorhynchus* Luhe, 1911. The present species differs from its closely related species in shape of body, arrangement of proboscis hooks, number of longitudinal rows and hooks, number of hooks per row and size of lemnisci. On the basis of aforementioned diagnostic differences, a new species *Centrorhynchus vulgari* is proposed. Previously there is no record of the genus *Centrorhynchus* from *Sturnus vulgaris*. However, this is the new host record for the genus *Centrorhynchus* Luhe, 1911.

**FIRST REPORT OF *PARYPHOSTOMUM RADIATUM* (DUJARDIN, 1845)
DIETZ, 1909 FROM PAKISTAN IN LITTLE CORMORANT, *PHALACROCORAX
NIGER***

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During the helminthological investigations of various avian hosts, 10 trematodes belonging to the genus *Paramphistomum* Dietz, 1909 were collected from intestine of 06 hosts. The trematodes in their native state were pressed under slight cover glass pressure, fixed in 70% ethanol, stained in Borax carmine, dehydrated in graded series of ethanol, cleared in clove oil and xylol. The specimens were finally mounted in Canada balsam. Diagrams were made with the aid of camera Lucida for detailed study. On the basis of number and arrangement of hooks, shape and position of testes, distribution of vitellaria and other morphological characteristics, the present worms are identified as *Paramphistomum radiatum* (Dujardin, 1845) Dietz, 1909. However, this species is being reported for the first time from Pakistan

**IDENTIFICATION OF PLANT PARASITIC NEMATODES (TYLENCHIDA) OF
EGGPLANT (*SOLANUM MELONGENA* L.) IN SISTAN REGIONS**

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To identify the nematode (Tylenchida) of eggplant in Sistan regions, 35 soil and root sample were collected during year 2009. Nematode extracted from roots by Coolen and D'herd nematode (Coolen and D'herd, 1972) and soils by the centrifugal flotation technique (Jenkins, 1964) and then they were fixed and transferred to glycerin following De Griss method (1969). The permanent slides were prepared from the extracted nematodes and some cross sections were made from different parts of the body. Morphological and morphometrical characters were studied by light microscope attached to a drawing tube. In this study 9 species belonging 7 genera of order Tylenchida were identified as follows: *Meloidogyne incognita*, *Meloidogyne javanica*, *Criconea mutabile*, *Helicotylenchus digonicus*, *Helicotylenchus pseudorobustus*, *Langidorus Proximus*, *Tylenchorhynchus maximus*, *Xiphinema pachtaicum*, *Paratylenchus similis*

INVESTIGATION OF PLANT PARASITIC NEMATODES FOUND ON OKRA IN SISTAN REGIONS

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In order to study the plant parasitic nematodes of okra (*Hibiscus esculentus* L.) in sistan regions about 18 soil sample were collected during year 2008. The nematodes were extracted by centrifugal flotation technique and then permanent slides were mounted and studied thoroughly (De Griss, 1969). In this study 6 species belonging 5 genera were characterized and identified which are as follows: *Aphelenchus avenae*, *Criconemella parvus*, *Ditylenchus medicaginis*, *Helicotylenchus vulgaris*, *Pratylenchus neglectus*, *Pratylenchus penetrans*.

CUTANEOUS LEISHMANIASIS IN SUSPECTED CASES WITH SPECIAL REFERENCE TO THE EFFICACY OF VARIOUS TREATMENT PROCEDURES

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The aim of the present study was to characterize the epidemiology of cutaneous leishmaniasis caused by *Leishmania tropica* and *Leishmania major* in Afghan refugees and local population of N.W.F.P Pakistan, the high probability of drugs use for leishmaniasis treatment and recognition of the diagnostic technique used for *Leishmania* identification. Leishmaniasis suspected population who visited Lady Reading Hospital Peshawar and Kuwait Teaching Hospital Peshawar were included in this study. A total of 320 suspected individuals were examined of which 223 were found positive for cutaneous leishmaniasis. Prevalence of leishmaniasis was higher in local population of N.W.F.P (73.06%) as compared to Afghan refugees (58.66%). The leishmaniasis was higher in males (77.83%) as compared to females (55.55%). The high prevalence was found in 0-9 age group (82.60%), on face region (71.77%) as compared to other parts of the body, this may be due to low immunity against leishmaniasis in children as compared to adults and face is the most exposed part for sand flies bite. The duration of lesion was recorded from 1-10 months and wet lesions dominated dry lesions. Microscopic diagnostic technique is cheaper than biopsy and culture and is widely available in each laboratory. Treatment by intra-lesional injection was found more effective than intra-muscular and oral treatment. Glucantime and Meglutin were used for intra-lesional and intra-muscular injections, while Flagyl was used for oral treatment.

**FIRST REPORT OF GENUS *ORNITHOBILHARZIA* ODHNER, 1912 FROM
PAKISTAN REPORTED IN AVIAN HOST**

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04 schistosomes belonging to the genus *Ornithobilharzia* Odhner, 1912 were collected from the mesenteries of the Little Cormorant *Phalacrocorax niger*. A total of 05 Little Cormorants were captured from Khairpur Nathan Shah, District Dadu, Sindh, Pakistan. Previously this genus is reported in avian hosts from Brazil, Canada, Czechosl. East Siberia, Europe, India, Italy, Japan, North Carolina, Sweden, U.S.A. and USSR. The present specimens can not be identified up to the species level due to the lack of some diagnostic structures. Therefore the present specimens are identified as *Ornithobilharzia* sp. This is first record of the genus *Ornithobilharzia* Odhner, 1912 from Pakistan.

**INCIDENCE AND INTENSITY OF SOME HELMINTH PARASITES IN SOME
FRESH WATER FISHES OF DISTT. THATTA AND SUBURBS**

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Fresh water fish belonging to four Families / genera i) *Channa* sp. (*Ophiocephalus*), ii) *Tilapia* sp., iii) *Mystus* sp. and *Wallago attu* were either caught through Netting near Thatta district, or bought from the local fish market-Karachi at random intervals. These were brought to the Laboratory for examination of Helminth Parasites, rate of incidence, intensity and percentage of infection of parasites per fish: (1) *Channa* sp. is a very common edible fish in Sindh especially in Karachi. This fish was found to harbor the well known Acanthocephala *Pallisentis* sp. in its small intestine. Total infection with male and female Acanthocephala parasite in 5 *Channa* sp. was 19 worms with 11 male and 8 females. Percentage of infection was 41.6 and intensity of infection was 1-5 worms per fish. (2) *Tilapia* sp. is an introduced fish, consumed mostly by the local people in Thatta and suburbs. This fish was found infected with larval nematodes. Four fish were examined and two were found infected, percentage of infection was 50% and intensity was 1-8 worms per fish. (3) *Mystus* sp. is commonly known as Catfish. This fish is also consumed locally out of twenty fish examined 6 were found infected with Nematodes of the genus *Contracaecum* sp. Percentage of infection was 30% and intensity was 1-6 worms per fish. (4) *Wallago attu* is a very common and delicious fish consumed very commonly in the city. The fish was found infected with

tapeworm of the genus *Gangesia*. Out of six fish Examined two were found infected percentage of infection was 33.3% and intensity of infection was 1-4 worms per fish.

COM PARATIVE PREVALENCE (%) OF *PEDICULUS HUMANUS CAPITIS* (HEAD LOUSE) INFESTATION BETWEEN PRIMARY AND SECONDARY SCHOOL CHILDREN ACROSS ALL DISTRICTS OF KARACHI

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Present study was conducted during the year 2006 - 2008. A total of 4025 students out of which 2000 Primary and 2025 Secondary school students, were examined through head combing for head louse infestation from 80 schools in Karachi. Overall infestation of head louse was found to be 25.3%. Infestation between primary and secondary schools differed significantly ($F=4.596$, $P<0.05$). Infestation in Primary schools (28.72%) was found to be higher as compared to the Secondary school children (21.90%). In Primary schools the rate of infestation across districts was found higher (37.25%) in District West and in Secondary schools, it was highest in District Central (30.35%), whereas the lowest rate of infestation in Primary schools was found in District South (19.75%) and in Secondary schools it was (17.5%) in District East. Average infestation of adult lice (0.26) and (0.25) were found almost equal in both Primary and Secondary schools respectively, whereas average infestation of nymph (0.30) and nit (0.15) were found higher in Primary schools than in the secondary schools. Average infestation of nymph was (0.15) and that of nit was (0.05) in Secondary schools.

***PROTOSPIRURA UNIDENTATA* SP.N (NEMATODA: SPIRUROIDEA) INFECTING NORWAY RAT *RATTUS NORVEGICUS* IN KARACHI, SINDH**

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During research studies a total of 70 rats (*Rattus norvegicus*) were trapped from empress market, Karachi at random intervals. These were brought to the laboratory and were autopsied for endoparasites examination. Out of 70 rats 60 rats were found infected with helminth parasites. The stomach was found highly infected with nematodes which were found penetrating in stomach wall and appeared hanging position in lumen of the

stomach. Six hundreds and ninety eight (698) specimens (263 male & 435 females) were recovered, these were preserved in 70% alcohol & glycerine. Detailed studies revealed that these belong to the genus *protospirura*, Seurat, 1914 and a new species *unidentata*. These are medium to large sized worm, brownish in color. Females larger and stouter than the males. Cuticle thick with transverse striations. Anterior extremity with highly develop pseud'olabia, raised above oral opening. Two lateral lips are very conspicuous and each is deeply trilobed. Each lobe is provided with single teeth on its internal surface. In an enface view the median lobe appears to be squarish in out line. In higher magnification, the surface of each lobe appears to be raised into elevations giving it a shape of an outer surface of a brain. Each lobe at its basal portion is provided with a signal large papilla, 4 pairs are an anterior dorsal surface, 3 pairs are on mid-dorsal surface and five pairs are on the inner-lateral surface of the labial lobes. In addition there are several pairs of minute papilla situated in the spaces in between the labial grooves. Pharynx thick walled and Esophagus divided into short muscular and a longer glandular portions. The new species is characterized by possessing cushion-shaped, stumpy labial lobes, each of which bears a single conical tooth or denticle. Vulva a little anterior to mid body and different body dimensions. Serve to propose a new species *P. unidentata*. Species name refer to the labial lobes bearing single denticle.

SECTION - V**FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER
BIOLOGY AND MARINE BIOLOGY****POPULATION DYNAMICS AND FOOD HABITS OF RANID FROGS IN THE
RICE-BASED CROPPING SYSTEM OF GUJRANWALA REGION**

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The present study aimed to investigate population dynamics and food habits of Ranid frogs in rice-based cropping system of Gujranwala District. The study area was divided into three different sampling units; each unit was further divided into three plots of one acre size each. For population estimation "Visual Encounter Survey" and "Capture, Mark, Release" methods were used. The food habits of the species were investigated by stomach contents analysis. Visual Encounter Survey showed a population of 52.71 ± 20.09 / ha at study site-I, 48.54 ± 16.79 / ha at site-II and 45.42 ± 16.15 / ha at site-III. In comparison, Capture, Mark, Release method showed relatively lower densities viz. 29.22 ± 12.39 / ha at site-I, 20.58 ± 6.19 / ha at site-II and 25.42 ± 9.01 / ha at site-III. Minimum population was recorded during winter 2008 while maximum in summer 2009 with a significant difference of $p < 0.05$. Analysis of stomach contents of frogs revealed eight different kinds of prey items in the study area; insects constituted 80.3%, earthworms 28.5 %, other frogs 15.8 %, bone pieces 22.5 %, rodents 1.66 %, vegetative parts 5.0 %, soil particles 13.3 % and some unidentified material 7.5 %. Most frequently consumed prey items were insects (80 % by volume). The frog species also occasionally consumed conspecific and rodents as their prey. Insects recovered from the stomach contents included Orthoptera, Lepidoptera, Coleoptera, Diptera, Arachnida, Odonata and Homoptera. Average number of insects recovered from the stomach contents were compared to those captured from the study sites by sweep nets and a strong positive correlation was found between Diptera ($r = 0.968$), Coleoptera ($r = 0.967$), and Orthoptera ($r = 0.867$).

TWO NEW RECORDS OF POLYCHAETES FROM PAKISTANI WATERS

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During a recent investigation of marine benthic invertebrates, several unreported polychaetes were observed. Of which two species are selected for the present paper. They are *Sternaspis scutata* (Ranzani, 1817) of Family Sternaspididae and *Euclymene oerstedii*

(Claparede, 1863) of Family Maldanidae. As far as we know they are not yet reported from Pakistani waters, the former is a cosmopolitan species while the latter extends from the Indian to Atlantic Ocean. Both the species are illustrated and described.

DETERMINATION OF FORAGE RATIO OF A CYPRINID FISH *CHELA LAUBUCA* (HAMILTON, 1822) IN LAKE HALEJI (THATTA, SINDH) PAKISTAN

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Studies on the population dynamics of algal flora and fish species in Haleji lake were carried out. The lake was observed rich in primary productivity in the form of planktonic unicellular, colonial and filamentous algal species. Total 73 species of phytoplankton were recorded. Among these 53.84% of Cyanophyta (represented by bloom forming species (*Anabaena*, *Coelosphaerium*, *Gomphosphaeria*, *Microcystis*, *Merismopedia*, *Oscillatoria* and *Spirulina*), 33% of Chlorophyta (*Ankistrodesmus*, *Botryococcus*, *Coelastrum*, *Crucigena*, *Oocystis*, *Pediastrum*, *Tetradron* and *Scenedesmus*) and 13.84% of Bacillariophyta (*Amphora*, *Cyclotella*, *Cymbella*, *Cocconies*, *Gyrosigma* and *Melosira*). 19 species of Zooplankton were identified from lake environment. Out of this, 15 belonging to phylum rotifera, 3 species of copepoda and 1 species of cladocera were dominant during the study period. 240 guts of *Chela labuca* were examined for six months (April-September 2008) 40 in each. It was observed that from the gut *Anabaena*, *Coelosphaerium*, *Gomphosphaeria*, *Microcystis*, *Oocystis*, *Pediastrum*, *Tetradron* and *Scenedesmus*, *Cyclotella*, *Cymbella*, *Cocconies* and *Gyrosigma* found to be dominant, while among zooplankton only one species of *Brachionus* was rarely found. It is concluded that the fish was found to be herbivorous in feeding habit.

POPULATION ESTIMATES, FOOD HABITS AND HABITAT PREFERENCE OF SMALL INDIAN MONGOOSE (*HERPESTES JAVANICUS*) IN POTOHAR PLATEAU, PAKISTAN

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The small Indian mongoose (*Herpestes javanicus*) is an important carnivore in biological niche, as it preys upon a variety of invertebrates, arthropods, small reptiles and mammalian species. In Potohar region, it is distributed in different habitats. The present

study was undertaken to glean information regarding the population, food habits and habitat preference of the species at six different study sites covering wild areas of Chakwal and Rawalpindi Districts. The population of the species was estimated using indirect method of burrows count and also by establishing foot tracks in the activity areas. The investigation of food habits was carried out by faecal pellet analysis, while the habitat preference of the species was studied by a comparison of three different habitats in the study area. The results revealed an average population density of 0.083 /ha in Chakwal region and 0.085 /ha in Rawalpindi region. Analysis of scat samples of the species revealed that the faecal matter on average (by volume) consisted of hairs (66.18%), bones (7.11 %), insects (10.54 %), plant parts (3.15 %), seeds (5.54 %) and some unidentified food items (6.98 %). The hairs recovered from the faecal samples of the two regions matched with the hair samples of *Rattus rattus* collected from the study sites. The most preferred habitat of the species was the type of habitat usually located in the vicinity of human habitation, also having some poultry farms. The study provides first scientific record about the important ecological aspects of the species in the Potohar plateau.

**ANALYSIS OF TRACE ELEMENTS IN FISH FROM THE INDUS RIVER,
PAKISTAN FOR ENVIRONMENTAL MONITORING AND FOOD SAFETY
ASSESSMENT BY ICP-OES**

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This study uses fish as an indicator of metal contamination in the Indus River of Pakistan and also for the evaluation of potential risks to human health by the ingestion of fish contaminated by metals. For this purpose manganese (Mn), copper (Cu), zinc (Zn), and chromium (Cr) were analysed in the gills, skin and muscle tissues of *Cyprinus carpio*, *Labeo rohita*, and *Oreochromis mossambicus* by inductively coupled plasma optical emission spectroscopy (ICP-OES). The study was arranged as a 3x2 factorial experiment by involving twenty seven specimens of each of the three freshwater fish species from each of the two sampling sites. The data were statistically analysed by using the Minitab software to compare the main effects of the fish species and the sampling sites and their interaction for each trace element. The gills generally had the highest trace elements concentrations, followed by skin and muscles. The overall order of Mn and Cr bioaccumulation in different fish tissues was gills>muscles>skin and muscle>gill>skin respectively, whereas order of Cu and Zn bioaccumulation was gills>skin>muscle. The fish species differed for the patterns of trace elements bioaccumulation which showed their variable ability to deal with the potential trace elements contamination of freshwater. The concentrations of Mn, Zn and Cr were higher (except Cu) in fish tissues than the acceptable values for food fish. This study highlights

the importance of monitoring fish for trace elements which could be harmful for the aquatic life including fish and consequently the potential health risks of human who consume fish.

SEASONAL VARIATION AND BIODIVERSITY OF FISH FAUNA OF KARACHI COAST

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A survey was conducted during March 2007 to September 2007 for the observation of biodiversity and seasonal variation of fish fauna of Karachi coast. During observation period overall 90 species were collected which belong to 64 genera 37 families of 11 orders. In the different months number of specimens and species are different. A large number of species belong to the order Perciformes whereas some species were collected throughout the collection period while most of the species were found only specific period.

CYPRINUS CARPIO AS A BIOMARKER TO MONITOR MINERAL POLLUTION IN THE INDUS RIVER IN THE MIANWALI DISTRICT OF PAKISTAN

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This study used *Cyprinus carpio* as a biomarker to monitor mineral pollution at two sites (CH, SK) around Indus River in Mianwali district of Pakistan. Water quality and fish tissues were investigated for the bioaccumulation of Na, K, Mg, Ca, Mn, Pb, Cu, Zn, Fe, Hg and Cr. The replicated study was arranged as either a completely randomised experiment to test the effect of sites on water quality and fish parameters or a 2x 4 factorial experiment to compare these sites and 4 tissues (Gills, Skin, Fins and Scales) for various biophysical, chemical and mineral profiles of these samples. The data were statistically compared for the main effects of the sampling sites on the water and fish parameters or the main effects of the site and fish tissues and their interaction on the bioaccumulation pattern of each mineral in fish tissues at $P < 0.05$. Significant differences between fish tissues and sites were observed for these minerals. The Indus River water was found to be suitable for aquatic life. However, variable mineral bioaccumulations in

different tissues suggested that these fish will require further investigations before their use for human consumption. The gills had the highest metal load followed by fins, skin and scales. Na, K, Mn, Cu, Zn, Fe and Cr concentration in different fish tissues were higher at downstream (CH) than upstream (SK) whereas Mg, Ca, Pb and Hg concentrations were higher at SK than CH site ($P < 0.001$). This study revealed that mineral concentrations were several fold higher in fish tissues than water samples; indicating the biomagnification of these metals in fish tissues through the aquatic food chain. It appeared that the most exposed parts of the fish could be used in the biomonitoring programmes of freshwater quality and perhaps as an early warning indicator for freshwater pollutions.

CAPTIVE CRANES, THEIR EGG HATCHING, DIET AND DISEASES IN SOUTHERN DISTRICTS OF NWFP, PAKISTAN

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A study was aimed to overlook on egg hatching, diet and diseases of common (*Grus grus* L.) and demoiselle (*Anthropokledes virgo* L.) captive cranes, in Southern districts of NWFP, Pakistan. Field survey, questionnaire and interview with communities were the major tools for the data collection. Total 165 and 85 camps were visited in fall 2008 and spring-2009, respectively. These camps were established in Baran Dam, Kurram, Kashu, Kethu and Dowa in Bannu; and Gambilla, Lunder and Chall rivers in Lakki. The numbers of hunters 1650 have 6600 demoiselle and 3300 common captive cranes in Bannu and Lakki. From 920 breeding pairs, 900 eggs were obtained, from which only 640 were hatched. Among natural foods of the cranes, snails, grasshoppers and earthworms, the pebbles were the most favorite food. The young ones of cranes were fed on maize bread, eggs of insects and other small animals, wasp's larvae and grasshoppers by hunters. They faced the problems of development of feathers, trapping in mud and parasitic attack during their development. The cranes suffered from many diseases; head tumor. Influenza and stomach blockage were the most common in the adults and young ones. The hunters used traditional things, garlic, coriander and brown sugar with antibiotics for treatments of different diseases. Migratory cranes are found to be declining viewed by hunters in southern districts of NWFP. Knowledge about egg hatching, foods and diseases of common and demoiselle captive cranes may assist in their conservation.

**LENGTH-WEIGHT RELATIONSHIP AND RELATIVE CONDITIONS OF
GENUS *HYPORAMPHUS* (FAMILY HEMIRAMPHIDAE) FROM KARACHI
COAST**

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In the present study, length-weight relationship and condition factors were estimated for the samples of 236 specimens of Genus *Hyporhamphus* caught from the Karachi coast from the Karachi Fish Harbour, during the monthly sampling from August 2009 - November 2009. In fishery research, length and weight measurements are standard tasks and data obtained are the backbone of many models used in fish population. Log transformed regression was used to test the growth of the specimens. Estimation of length-weight relationship in *Hyporhamphus* species justifies the equation for each month as, August, male: $\text{Log } W = -14.51 + 1.85 \log L$; female: $\text{Log } W = -13.35 + 1.51 \log L$; September, male: $\text{Log } W = -39.12 + 3.12 \log L$; female: $\text{Log } W = -32.35 + 2.71 \log L$; October, male: $\text{Log } W = -36.96 + 2.92 \log L$; female: $\text{Log } W = -24.51 + 2.33 \log L$; November, male: $\text{Log } W = -49.32 + 3.55 \log L$; female: $\text{Log } W = -28.47 + 2.58 \log L$. The parameters 'a' and 'b' of the length weight relationship of *Hyporhamphus* in the form of $W = aL^b$ was determined. It was observed that the growth is allometric as the value of coefficient 'b' is greater in males as compare to females. The value of "b" varied between 1.51 and 3.55. The overall mean value of "b" was 2.50 for the complete set of data. The condition factor (K) was ranged from 0.26 - 0.40 (mean of 0.30) in males and 0.26 - 0.28 (mean of 0.27) in females. The relationships (linear regression) was estimated as 0.97 - 0.99. It concluded that the growth of fish or body proportions of fishes changed with their growth.

**POTENTIAL AND PROBLEMS ASSOCIATED WITH THE ENDEMIC FLORA
OF PAKISTAN**

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Pakistan is unique of having wide range of altitudinal variations from the sea level to the second highest peak K2 (8611m). It provided home to some of the world's ancient civilizations and has the junction of the world's famous mountain systems Himalaya, Hindu Kush and Karakoram. Review of the available information shows that Pakistan has 372 endemic species distributed in various parts of the country, from sea shore to the alpine meadows. A sum of 325 species belonging to 48 families is taken as content of this paper. Among which *Papilionaceae* is at top with 65 species, followed by *Boraginaceae*

(26), *Brassicaceae* (21), *Poaceae* (19), *Ranunculaceae* (17) and *Labiatae* (15). Among the genera, *Astragalus* alone is represented by 39 endemic species therefore stands 1st with respect to endemism. Phytogeographic regions having higher number of endemics are identified as Irano-Turanian followed by Sino-Japanese and Sharo-Sindian regions of the country. Some of the hot spots identified regarding the distribution of endemics are Chitral, Kashmir and Gilgit-Baltistan areas. This paper communicates the information gathered about the distribution, conservation status, threats and strategies to conserve the gene pool of these endemic taxa.

STUDY ON FEEDING HABITS OF BARN OWL (*TYTO ALBA*) AND SPOTTED LITTLE OWL (*ATHENE BRAMA*) INHABITING AHMADPUR SHARQIA, DISTRICT BAHAWALPUR, SOUTH PUNJAB, PAKISTAN

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The present study was carried out in Ahmadpur Sharqia, District Bahawalpur, Punjab to investigate the feeding habits of two owl species; Barn Owl (*Tyto alba*) and Spotted Little Owl (*Athene brama*). Two study sites were selected after conducting detailed survey of old tree grooves, ruins and buildings in the urban and suburban areas of Ahmadpur Sharqia. Nests of Barn Owl were found at the top roof of Jamey Masjid Muhammadabad, while for the Spotted Little Owl were located in the premises of Govt. Sadiq Abbas Degree College, Dera Nawab Sahib. A total of 946 pellets of the two species were collected from February 2008 to January 2009; 840 of Barn owl and 106 of Spotted Little Owl. Average weights, average length and breadth of all the pellets were recorded. The analysis of the pellets showed that the diet of Barn Owl comprised mainly of small mammals (81.4%), birds (13.9 %), insects (2.1%) and unidentified items of prey (2.5%). The best consumed prey throughout the year was house shrew (56.1 %). The rest of the mammalian prey included *Mus musculus* (6.1 %), *Rattus rattus* (3.3%), *Mus booduga* (0.6%), *Milliardia meltada* (0.9%), *Bendicoota bengalensis* (0.3%) and Bats (13.8%). Birds were the second best consumed food of Barn owl; including Common Myna (9.9 %), House Sparrow (3.7%), Dove (0.1 %), and Cuckoo (0.1 %). The diet varied in different seasons. The diet of Spotted Little Owl consisted of small mammals (28.6%), House Sparrow (3.8%), lizard (5.9%), frogs (1.6%), insects (44.9%) and unidentified plant and animal material (15.1 %). Insects were the best consumed food throughout the year. *M. musculus* (17.8%) was the second choice, followed by lizards (5.9%) and bats (5.4%). Small mammals appeared regularly in the diet whereas insects

were consumed throughout the year. The results conclude that Barn Owl . preferably feeds on house shrews, although it also consumes birds, bats and rodents in a good number, while Spotted Little Owl mostly prefers insects, although it also consumes small mammals in a good number.

FACTORS AFFECTING THE SHRIMP ASSEMBLAGE IN SIX STREAMS OF KIKAI-JIMA ISLAND, SOUTHERN JAPAN

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Present study aimed to investigate the effect of altitude on the composition and assemblage of freshwater shrimp fauna in Kikai-jima Island, one of the Ryukyu group of Islands. In total six stations, Karimata-no-izumi, Shiomichi, Nagamine, Takigawa, Ooasato and Urabaru, comprising of stream pool stations were sampled during June 2005 through June 2008. Eleven species, ~elonging to two families, four in Palaemonidae and seven in Atyidae were identified. Family atyidae; *Caridina typus*, *C. sakishimensis*, *C. multidentata*, *C. grandirostris*, *C. leucosticta* and *P. compressa* and Palaemonidae; *Macrobrachium lar*, *M japonicum*, *M. formosense* and *M. australe*. Insular shrimps are subjected to investigate the dispersion and biogeography. Altitude is one the the key determinate of the species composition in streams. Low altitudes may play the role in early and successful recruitments of juveniles of amphidromous species. High shrimp diversity H' was calculated in the stations located at low altitudes and low diversity was recorded at high altitudes. Species diversity was inversely related to altitude with a high coefficient of correlation between diversity index H' and station altitude ($r^2 = 0.83$). Consequently, altitude life cycle of the shrimp species and the drainage system were the main factors responsible for shrimp assemblage and diversity in the study sites.

FEEDING HABITS OF TWO SPECIES OF *MUTATA* (CRUSTACEA: BRACHYURA: MUTATINAE) FROM KARACHI COAST

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The food habits of brachyuran crabs *Mutata lunaris* and *M. platipes* were investigated using the stomachs of 98 crabs collected from April, 2009 to December,

2009. Stomach contents of 98 crabs from each season were examined. The stomachs of all 98 crabs collected were dissected in the dietary components identified to the taxonomic level possible. Fifteen taxonomic categories were identified from the 980 prey items analyzed. The main food items included crustaceans such as barnacles, shrimps, small crabs, mollusks; bivalves, gastropods, pelycepoda species, chordates; small fish bones, scales and vertebrae, algae, sea weeds, echinoderms; star fish and brittle star, organic debris and dead animal tissues. Of the prey categories crustaceans were more important than the others. The proportion of food items was observed in crabs of carapace ranged from 26-mm to 51 mm. Exploitation of different food items and selective feeding on common prey may reduced inter specific competition for natural food resources and allows the 531 crabs to co-exist in the same habitat. The main food items include molluscs (34.23%), crustaceans (36.27%), fish bones (29.31%), polychaetes (11.99%) and organic debris (21.41%). There was no significant difference in the frequency of occurrence of food items among size or according to season. The importance of vertebrates specially fish in the diet of the crabs increased with increasing body size of crab. But at the same time the importance of invertebrates decreased. Both the stomach fullness (a subjective estimation) of the food investigated was recorded.

REVIEW OF FISHES BELONGING TO THE FAMILY LUTJANIDAE (ORDER PERCIFORMES)

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The present study was based on the literature review of the fishes belonging to the snapper family Lutjanidae. This family contains 17 genera and 103 species. Data parameters about the morphology and early life history pattern not measured during the survey were taken from the FishBase data base (see www.fishbase.org and Froese and Pauly, 2000). These include Lmax (Maximum length), Linf (Length infinity); K (growth rate); M (natural mortality); LS (Life Span); Lm (Length at maturity); tm (Age at first maturity); to (Age at zero length); tmax (Longevity) and morphological data also. Morphological and life history data were taken only for five species of fishes belongs to genus *Lutjanus* i.e. *L. johnii*, *L. malabaricus*, *L. lutjanus*, *L. fulvus*, *L. russellii*. About 270 samples were collected from Karachi fish harbor during period January 2007-December 2008. The main objective of this study was to review some aspects of distribution, morphology and feeding habits, spawning and also acquired and analyzed information on selected life history variables to described patterns of variation among different species of snappers.

**LENGTH-WEIGHT RELATIONSHIP, CONDITION AND RELATIVE
CONDITION OF LUTJANID FISHES (PERCIFORMES: LUTJANIDAE) FROM
KARACHI FISH HARBOR, PAKISTAN**

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Fishes of the family *Lutjanidae* (genus *Lutjanus*) collected from Karachi fish harbor were studied with reference to their length-weight relationship and condition. About 370 specimens of different species of genus *Lutjanus* of family *Lutjanidae* collected during the period June 2007 to November 2008, were used for the present studies. Length- Weight parameters for the different species of family *Lutjanidae* males and females as well as combined sexes were given. The values of the exponent (b) for the males~ females and combined sexes belong to same species were found to be different. Results of length-weight relationship studies showed the applicability of the cub law. Species of family *Lutjanidae* exhibited both allometric and isometric pattern of growth. The average value of the relative condition factor (Kn) was found to be one (except *L. russellii*) indicating that fishes were in good condition.

**CAPACITY BUILDING FOR SUSTAINABLE DEVELOPMENT OF OCEAN
RESOURCES**

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Extensive research and development in marine sciences and the interrelated disciplines of Oceanography have become increasingly important for us in recent years. As we become more aware of the benefits that can be derived from the coastal and offshore maritime areas. The fascination with the marine sciences continues to grow as we find new resources such as economically important minerals embedded in sea floor and offshore gas hydrates as potential new energy sources and food from the oceans to supplement the depleting land resources. The ocean has a vast wealth of resources that could contribute towards national economic growth and development through the creation of innovative technology for aquaculture of commercially viable marine species as a new income generating source. Introduction of new fishing and seafood processing technologies, better management of ports and harbors, tourism and recreational sports such as fishing and diving have become essential as we continue to find alternate ways for sustainable income generation for coastal communities. With the increasing incidences of natural phenomenon such as tsunamis, earthquakes, volcanic eruptions and

atmospheric disruptions, marine sciences has become even more important. Marine scientists and oceanographers have for decades looked to the oceans to develop a better understanding of the global weather patterns and natural phenomena's that have a directed bearing on our living and non living resources. The paper examines the need for capacity building for the development of ocean resources that can contribute towards national economic growth and progress.

LENGTH-WEIGHT RELATIONSHIP AND RELATIVE CONDITION FACTOR (Kn) OF PALRI, *GUDUSIA CHAPRA* (HAMILTON) FROM THE BACKWARD CHANNEL OF RIVER INDUS NEAR MANJHAND

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Length-weight relationship and relative condition factor (Kn) of palri, *Gudusia chapra* (Hamilton) from backward channel of River Indus near Manjhand were studied from 251 specimens (140 and 111 male and female respectively) ranging from 69 to 182 mm (TL) and 2.24 to 20.65 g in weight. The equations of length-weight relationship were:

$\text{Log } W = -1.18 + 2.05 \times \text{Log } L$ for male

$\text{Log } W = -2.32 + 2.25 \times \text{Log } L$ for female

$\text{Log } W = -1.90 + 2.15 \times \text{Log } L$ for combined sexes.

The relationship was significant at 0.1 % level in data indicating allometric growth pattern. Relative condition factor (Kn) values were ranged from 0.77-1.08, 0.89-1.10 and 0.88-1.09 in male, female and combined sexes respectively. Maximum relative condition factor values were observed in smaller sized fishes. The length, weight relationship and condition factor values indicated that the growth of palri, *Gudusia chapra*, from backward channel of river Indus near Manjhand, termed as satisfactory.

EFFECT OF DIFFERENT PROTEIN LEVEL ON GROWTH AND SURVIVAL OF THE *CATLA CATLA* (HAMILTON) REARED IN GLASS AQUARIA

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To study the effect of different protein levels on growth and survival of *Catla catla* (Hamilton) reared in glass aquaria during May to August 2008. Three iso-caloric feeds were formulated with locally available ingredients (rice protein, rice bran and wheat bran) of different protein levels such as 30%, 35% and 40% (dietary protein levels)

of 2 mm dia were prepared with the help of manually operated pellet machine. The feed ingredients were tested for proximate (bio-chemical composition) analysis according to the methods given in AOAC (1980) and found 13%, 12% and 40% protein respectively. Each feed was supplied at a rate of 8% of the body weight of fish twice a day. The results of the various growth parameters like suitability of protein level requirement, specific growth rate, mean total weight gain, percentage weight gain, feed conversion ratio, survival rate and production of the experimental fish showed significantly ($p < 0.05$) highest growth and production was observed in feed B followed by feed C while significantly ($p < 0.05$) lowest growth and production was recorded from feed A. It is therefore concluded that the feed with 35% gross protein found to be suitable for the better growth and production of major carps.

**EFFECT OF STOCKING DENSITY ON GROWTH AND SURVIVAL RATE OF
LABEO ROHITA (HAMILTON) REARED IN GLASS AQUARIA FED WITH
FORMULATED FEED**

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To study the effect of stocking density on growth and survival rate of *Labeo rohita*, a three month experiment was conducted during July to September 2009. Three different densities 10, 15 and 20 Fish/aquaria were assigned as treatment I, II and III, respectively. The experimental fish were fed with 35% (gross protein) formulated feed. The result of the density experiment showed significantly ($p < 0.05$) highest growth and survival rate was obtained in treatment II (15 fish/aquaria), while significantly lowest growth and survival was recorded in treatment III (20 fish/aquaria).

**POPULATION ESTIMATION AND HABITAT EVALUATION OF TWO
PARTRIDGE SPECIES AT LEHRI NATURE PARK, DISTRICT JHELM**

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The present research deals with estimation of population densities of two partridge species; grey partridge (*Francolinus pondicerianus*) and black partridge (*Francolinus francolinus*), at Lehri Nature Park, District Jhelum, Punjab. The study was carried out from September 2008 to July 2009. The study area was divided into three different

sampling sites, on the basis of different habitats. Variable Width Line Transect method was used. The average density of partridges in habitat-I was estimated to be $0.79 \pm 0.21/\text{ha}$ (maximum), in habitat-II $0.46 \pm 0.06/\text{ha}$ (comparatively lower), while in habitat-III $0.39 \pm 0.07/\text{ha}$ (minimum). Mean densities of partridges were significantly different ($P \leq 0.05$) in different habitat types. A significant difference ($p < 0.05$) was also found between the morning and evening time populations. Major plants species at the three selected habitats were identified and relative density, relative frequency and relative dominance, for trees, shrubs and herbs species were calculated. It was found that habitat-I was the most preferred habitat and it supported maximum population ($0.79 \pm 0.21 / \text{ha}$) of the partridge species. The current study revealed a valuable correlation between partridge populations and habitat type.

BIRD DIVERSITY AND CONSERVATION AT KALLAR KAHAR LAKE WITH SPECIAL REFERENCE TO WATER BIRDS

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Kallar Kahar Lake is located in semi-arid hill ranges in the north-central part of Pakistan. Present study was conducted to document richness, density and relative abundance of avifauna of the lake particularly the water birds. Data were collected through observations at the lake using binoculars and spotting scope, and birds were identified using field guides. A total of 86 bird species belonging to 16 orders and 36 families were recorded. Among these, 46 % species were residents, 25.58% winter visitors, 8.13% summer breeding visitors, 4.56 % passage migrants, and 1.16% feral. Water birds constituted 33.72% of avifauna of the lake. As many as 462 individuals of 23 species of water birds (excluding ducks) were recorded with black coot (*Fulica atra*) as the most abundant species. As many as 271 individuals of six species of ducks were recorded with Shovelar (*Anas clypeata*) as the most abundant duck. The densities of water birds and ducks were estimated to be 3.44 and 1.99 birds per hectare, respectively. It was estimated that presently around 31% (42 ha.) of lake area has been invaded by *Phragmites*. It provides breeding sites to night heron and little egret having ± 189 and ± 99 nests, respectively. The estimated number of house crows and common mynas roosting in the vegetation is ± 6720 and ± 4200 , respectively. The lake as a whole depicts an alarmingly deplorable ecological condition which is evident from the 39.32 % reduction in the area of lake within a short span of seven years. Unregulated tourism, land encroachment, eutrophication and siltation are major threats to the lake. The ecological health of the lake and habitat quality for birds can be improved by zonation (wildlife and tourist's zone etc.), construction of check-dams to minimize the influx of silt and

nutrients, initiation of vegetation control program, strict check on land encroachment, regular wildlife research and monitoring programs and formulation of species recovery plan for significant species of the area such as white-headed duck.

IMPACT OF NEW DEVELOPMENTS ON MIGRATORY DUCKS AT RAWAL LAKE

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A total count of migratory ducks from November 2008 to July 2009 at Rawal Lake, Islamabad, was undertaken. Richness, relative abundance and density were calculated. The results were compared with past available counts of migratory birds at the lake. Out of 11 duck species reported from the lake (1983 through 2005), only seven species were recorded during the present study and a decline of around 38.38% in the abundance was recorded. It was concluded that increased human activities such as boating, fishing and tourism and developments such as construction of Lake View Park and Bani Gala Tourist Spot had affected richness and abundance of migratory ducks. As many as 585 individuals of seven species of migratory ducks were recorded with mallard (*Anas platyrhynchos*) (n=239) as the most abundant while gadwall (*Anas strepera*) (n=2) the least abundant species. Maximum number of ducks (n= 880) was recorded in the second week of January, 2009 while minimum number (n= 263) was recorded in the second week of March, 2009. Few ducks recorded by earlier workers such as wigeon (*Anas penelope*), garganey (*Anas querquedula*), red-crested pochard (*Netta rufina*), scaup (*Aythya marila*) were not observed during the present study.

POPULATION DENSITY OF KASHMIR FLYING SQUIRREL (HYLOPTES FIMBRIATUS) IN DHIR KOT, AZAD JAMMU & KASHMIR

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The Present study was conducted to estimate the population density of Kashmir flying squirrel (*Hylopetes fimbriatus*) in a thousand hectares forest area of Dhir Kot, AJK from October 2008 to June 2009. The study area was divided into two units, and line transect method was used. Each site was visited twice a month. Population density of Kashmir flying squirrel (*Hylopetes fimbriatus*) was found to be 2.28 squirrels per hectare at the study site. Population densities at site A and site B were estimated to be 2.23 and 2.33 per hectare respectively. Density of Kashmir flying squirrel (*Hylopetes fimbriatus*)

increased in summer months and declined in winter. Kashmir flying squirrel preferred to live in old, thick, and mature forest because of availability of more nesting and roosting sites. It was found that logging had negatively affected population density of flying squirrels.

ASSESSMENT OF FISH LOSS DUE TO AVIAN PREDATORS AT FISH FARMS IN GUJRANWALA

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Food crisis has become the global issue of latest times. Unprecedented human growth compels man to develop different methods such as fish farming to supplement his food requirements. These facilities attract a number of birds that feed upon fish, therefore, badly affecting the annual fish production. Present study was designed to document diversity of avian predators, and to investigate the loss of fish production caused by them at some selected fish farms in Gujranwala District from November 2008 to July 2009. Around six predatory bird species belonging to families Ardeidae, Alcedinidae and Phalacrocoracidae were found to prey upon fishes at the fish farms. Cormorant and kingfisher species were more nuisance for fisheries. Other bird species recorded from the study site that presumably preyed on fish or devoured carcass and other refuse of the farms included little bittern, night heron, black-headed yellow wagtail, white-headed yellow wagtail, black-winged stilt, black drongo, house sparrow and house crow. Mean percent fish loss was found to be 4.08 %. Annual fish loss was estimated to be 5.40%. Maximum and minimum monthly fish losses were found to be 6.09 % in February, 2009 and 2.28% in June, 2009, respectively. Rahu (*Labeo rohita*), gulfam (*Cyprinus carpio*) and silver carp (*Ctenopharengodon idella*) were found to be the most preferred fish species preyed by birds during the study period. Some measures such as frightening devices, barriers such as wire grid systems and netting, direct shooting of birds and modification of avian behavior like roosting or feeding locations through dispersal or hazing were suggested to reduce the fish loss.

OBSERVATION ON THE AVIAN FAUNA OF SAFARI PARK AND KARACHI UNIVERSITY CAMPUS, KARACHI

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Avifaunas are excellent natural indicators of ecosystem. One year study was carried out from January 2009 to December 2009 to estimate the population of birds on

Karachi University Campus and Safari Park areas. During this study, 37 species of birds belonging to 31 genera, 26 families and 9 orders were recorded in Karachi University Campus and 34 species belonging to 29 genera, 26 families and 10 orders were recorded in Safari Park. The areas were regularly visited in all seasons during the above mentioned period. The birds were identified using spotting scopes and binoculars.

**SOME OBSERVATIONS ON POPULATION OF GREY PARTRIDGE
(*FRANCOLINUS PONDICERIANUS*) IN AGRO-ECOSYSTEM OF POTHWAR
AREA**

ASMAT-UN-NISA AND IFTIKHAR HUSSAIN

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Grey partridge or grey francolin (*Francolinus pondicerianus*), a widely distributed game bird of Pakistan is better adapted with arid conditions/habitats of the country. The present study was conducted in a representative habitat of this species; the Pothwar area comprising of rain-fed agro-ecosystem and related scrub forest. The population was observed for habitat preference, population estimation and related biological aspects. The population density was estimated by direct sighting and was estimated to 13.6 ± 3.44 birds per hectare in cropland habitat and 6.49 ± 1.74 birds per hectare in forest habitat showing a significant preference ($t=2.51$, $df=8$ and $p<0.05$) for the agricultural tracks. This high density in agriculture was associated with the variety of crops in different seasons *i.e.* *Brassica campestris* (sarsoon), *Tritium aestivum* (wheat), *Lens culinaris* (masoor) and *Eruca sativa* (taramira). Field observation of the bird nests showed that breeding period was spanned from March to May. The data of 6 nests under study during the breeding season revealed that nests were found on ground in thick vegetation cover. The eggs were elongated-oval in shape and pale-brown in color. The average clutch size was 7.0 ± 0.36 eggs. The estimated incubation period was 20.6 ± 0.50 days (range 19-22 days), hatching success was 75.39% (5.33 ± 1.22 eggs), the average number of fledglings obtained was 3.83 ± 0.83 youngs with survival rate of about 63.08%.

**MORPHOMETRIC STUDY OF CATFISH, *RITA RITA* (HAMILTON) FROM
CEMENTED CISTERNS OF UNIVERSITY OF SINDH, JAMSHORO**

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Morphometric and meristic count of catfish, *Rita rita* (Hamilton) from stock maintained in cemented cisterns of University of Sindh were carried out from 120 specimens of total length (TL) ranging from 180 to 417 mm. Morphometric variables of

total length, standard length, head length, eye diameter, dorsal fin length, dorsal spine length, pectoral fin length, spine length, pelvic fin length and body girth. It was observed that all these above parameters showed linear relationship with total length while eye diameter found to be highly correlated with the head length. It was concluded that the growth of all the above mentioned parameters showed positive allometry growth pattern in catfish.

EFFECT OF FOOD AND NOISE STRESS ON BREEDING AND NON BREEDING DOMESTIC PIGEONS (*COLUMBA LIVIA DOMESTICA*)

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Domestic pigeons (*columba livia domestica*) breed throughout the year. About 10 days after mating, the female pigeon laid eggs. Clutch size in domestic pigeon is two eggs per clutch. The number of eggs per clutch varies from bird to bird and it depends upon the food availability and ambient temperature. Both male and female pigeon incubate the eggs. The eggs hatch about 18 days after incubation. Depending on ambient temperature, however, this may vary by a day or so either way. This study was carried out to investigate the effect of food and noise stress on breeding and non-breeding domestic pigeons. To determine the effect of these stresses 20 pairs of domestic pigeons were kept in captivity. In case of breeding group, during the breeding interval from June to November, 15 pairs bred on 32 occasions. The overall percentage of mating pairs, during the breeding interval was 71.1%. The total number of eggs laid during the breeding interval were 63 eggs, with the percentage of eggs laid during the breeding interval was 70%. Out of the 63 eggs only 47 eggs hatched, hence overall percentage of hatched eggs was determined 74.6%. Out of 47 chicks only 37 chicks survive, hence the overall percentage of chick survival was 78.7%. Variations observed during the breeding interval was due to food and noise stress because these stresses affect the breeding behaviour of the birds. In addition to breeding the food and noise stress also affect the feather growth formation of the birds. In case of non-breeding group, 5 pairs were taken to observe the effect of food and noise stress on feather growth formation. In case of food stress group, 9 birds (one bird died) showed total 134 fault bars/primaries at three different food levels (20%, 40%, 50%) during three different moulting periods. While, these birds showed total 310 fault bars/retrices at three different food levels (20%, 40%, 50%). In case of noise stress group, 9 birds showed total 41 fault bars/primaries at 700Hz frequency. Whereas, the total number of fault bars/retrices were 73.

**NITRATE CONTAMINATION IN DRINKING WATERS IN PUNJAB: AN EYE
OPENER FOR ENHANCED HUMAN HEALTH RISK**

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Nitrate contamination has recently been evaluated a growing human health risk in all age groups which is the main cause of stomach and alimentary canal cancer along with other disorders. Thereby, an extensive study was aimed to investigate the nitrate concentration of drinking waters of Punjab province, Pakistan. The drinking water from different sources (WASA water, individual pumps of medium depths, individual pumps of shallow depths, tube well waters and bottled mineral water) were collected from 36 districts of the Punjab Province. The samples were collected from different locations including rural and urban areas of each district. A sum of 1,480 water samples were collected during winter and summer seasons in order to overcome the seasonal bias. The water samples were adequately analyzed for nitrate concentration. The nitrate contamination ranged from 1-110 ppm. About 48% of water samples were found below the WHO permissible limit for drinking water, while 52% were noted above the safe limit. The study also revealed that shallow depth water sources were prone to higher nitrate contamination than deeper sources, and putting the people on enhanced risk of nitrate pollution and adverse health issues. It was also noticed that in the areas where sewerage water is passing by or intensive cultivation is practiced, more nitrate contents were determined in drinking waters close to such vicinity. There is an urgent need for massive awareness campaign about nitrate contamination and its potential adverse effects on human and animal life, in order to reduce the risk of cancer and other nitrate linked disorders.

**MORPHOMETRIC STUDY OF FEMALE POPULATION OF WILD
NOTOPTERUS NOTOPTERUS IN RELATION TO BODY SIZE AND
CONDITION FACTOR**

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Fifty four samples of variable size ranging from 18.5-29.5 cm total length and 47.80-219.38 g body weight were obtained from Indus river near Ghazi Ghatt a village near D. G. Khan. These samples were selected for the analysis of weight-length relationship. The log transformed regressions were used to test the allometric growth. Each fish was measured and weighed. It was observed that growth in weight is almost proportional to the cube of its length. The value of the slope b is 3.23, which is greater

than the slope of that of an ideal fish. Condition factor 'K' when plotted against total length and wet body weight shows an increasing trend with increasing wet body weight and total length. Highly significant correlations were found between log transformed data of all the parameters except pectoral fin weight that showed less significant correlation versus log total length. Highly significant correlations were found between log transformed data of all the parameters except pectoral fin weight versus log wet body weight. The regression coefficient 'b' for all the parameters against log wet body weight was found to be negative allometric. Highly significant correlations were found between log transformed data of all the parameters (except log dorsal fin base and log anal fin length) versus log total length. The regression coefficient 'b' was found to be negative allometric for all parameters except log anal fin base and standard length that showed positive allometry. Highly significant correlations were found between log transformed data of the parameters (log stomach, liver, heart, intestine, air bladder and gonad weight) versus log wet body weight. Significant correlation was found between log eye ball weight versus log wet body weight and non significant correlation was found between log kidney weight versus log wet body weight. The regression coefficient 'b' for the parameters against log wet body weight was found to be negative allometric (log stomach, intestine, eye ball, liver, kidney and gonads weight); positive (log heart and log air bladder weight).

**RESPONSE OF *CATLA CATLA*, *LABEO ROHITA* AND *CIRRHINA MRIGALA*
DURING SUB-LETHAL CHRONIC EXPOSURE OF IRON**

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Fish viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were exposed to sub-lethal concentration of iron under laboratory conditions. 60-day fish of each species were divided into two groups, One kept unstressed as a control while the other was exposed to sub-lethal iron concentrations of 33.33, 16.67 and 33.33 mg L⁻¹ for *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*, respectively, for a period of 30 days. The fish were dispensed with a feed (35% Digestible Protein and 2.90 K cal/g Digestible Energy) through out the experimental period. Limnological parameters such as water temperature, dissolved oxygen, pH, electrical conductivity, carbondioxide, total hardness, calcium, magnesium, total ammonia, sodium, potassium and chlorides were studied and recorded on daily basis. During this experimental period *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* showed average wet weight increment of 0.06, -1.11 and 0.62 g, respectively. Fish growth parameters such as wet weight, fork length, total length and feed intake were analyzed by using factorial experiment and Duncan's Multiple Range Tests by using three replicates, each.

DETERMINATION OF POPS IN THE WATER AND FISHES OF GALPANI NULLAH (MARDAN)

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The present study has been designed to determine the level of persistent organic pollutants in water and fishes of Galpani Nullah, Mardan, NWFP. The organic solvent (POPs) were also determined in water samples of different sampling sites and their concentration varied as DDD 0.26- 0.70 µg/l, DDE 0.37-0.72 µg/l, 2,4 DDT 0.31-0.74 µg/l, 4,4 DDT 0.29-0.67 µg/l, Total DDT 1.37-2.56 µg/l, B-HCH 0.135-0.730 µg/l, Lindane 0.256-0.677 µg/l, Heptachlor 0.288-0.688 µg/l, H-exo 0.254- 0.657 µg/l, H-endo 0.291-0.646 µg/l and Dieldrin 0.364-0.730 µg/l and fish species DDD 0.307- 0.827 µg/g, DDE 0.253-0.957 µg/g, 2,4 DDT 0.423-0.950 µg/g, 4,4 DDT 0.453-0.927 µg/l, Total DDT 1.447-3.210 µg/l, B-HCH 0.184-0.541 µg/l, Lindane 0.241-0.596 µg/l, Heptachlor 0.162-0.634 µg/l, H-exo 0.278- 0.583 µg/l, H- endo 0.278-0.540 µg/l and Dieldrin ND.

SEASONAL DYNAMICS, SLOPE ASPECT AND LAND USE EFFECTS ON SOIL FAUNAL POPULATION DENSITY AND DIVERSITY IN THE MID-HILLS OF CENTRAL HIMALAYA

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The study addressed the integrated effect of seasons, slope aspect and land use on soil faunal population density, diversity and QBS-ar in the mid-hills of the central Himalaya, Nepal. One of the objectives was to determine the relationship among soil biological and physio-chemical indicators. Some investigated physio-chemical variables were pH, moisture, temperature, texture, bulk density and organic carbon of the soil. Soil samples were collected from two different land use type: agriculture and forest in pre-monsoon (April) and post monsoon (October) in 2009 in the middle position of both north facing and south facing slopes. Soil core sampling (10*10*10) was performed, and fauna was extracted by using the modified Berlese Tullgren funnel. Particle size analysis was carried out using the soil hydrometer method, soil pH was measured using a pH probe with glass-calomel electrode and 1:1 soil: water ratio, soil organic matter and Soil

organic carbon was measured by Dry combustion method, soil temperature using a digital thermometer, soil moisture was determined by gravimetric method and bulk density was determined using the core method. Significant differences in soil biological and physio-chemical indicators with respect to seasons, land use and slope aspect were determined using three way factorial ANOVA. Pearson's correlation was done to determine the relationship among the soil physiochemical and biological indicators. Most of the physio-chemical properties differed significantly with respect to the land use, slope aspect and seasons. Soil faunal population density was highly statistically significant with the seasons ($p=0.000$) and land use ($p=0.009$), but non-significant with the slope aspect. Average faunal population density was higher in post monsoon (11245 individuals/m²) as compared to pre-monsoon (3765 ind./m²). Average faunal population density on both seasons was higher on north slope (pre monsoon: 4400 ind./m²; post monsoon: 12660 ind./m²) than southern slope (pre monsoon: 3130 ind./m²; post monsoon: 9830 ind./m²). Faunal density was higher in the forest as compared to agricultural land in both seasons. Shannon Diversity Index was weakly significant ($p=0.05$) with the seasons while QBS-ar was highly significant different ($p=0.000$) with the seasons. Pearson's correlation indicated that soil moisture was highly correlated ($p=0.01$) positively with sac and population density while negatively with soil temperature ($p=0.010$ in pre-monsoon. In post monsoon Bulk density was negatively correlated ($p=0.05$) with QBS-ar and population density ($p=0.05$). Results indicated that season, slope aspect and land use all had significant effects on soil biological and physio-chemical indicators. Soil moisture, temperature, soil organic carbon and bulk density appeared to be good indicators of soil quality and these factors also determine the density and diversity of soil fauna.

BIOASSAYS FOR THE EVALUATION OF SOME ENTOMOPATHOGENIC FUNGI AGAINST NYMPHS OF *BEMISIA TABACI* GENN. (HOMOPTERA: ALEYRODIDAE)

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The Whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) is an important cosmopolitan damaging insect pest in tropical and sub-tropical areas in agricultural and horticultural production systems. In Pakistan, it has a long history of devastating field crops specially cotton in different ways. The myco-insecticides are considered as most effective biological control agents. Therefore, the present studies were carried out to determine the efficacy and pathogenicity of some strains of entomopathogenic fungi viz., *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* against whitefly nymphs in Department of Entomology at University of Agriculture, Faisalabad

during spring 2008. Three different concentrations *i.e.*, 1×10^6 , 1×10^7 and 1×10^8 conidia mL^{-1} of each fungal strain were used against each nymphal stage using leaf immersion method. All tested conidial concentrations of *P. fumosoroseus* and *V. lecanii* strains were pathogenic and highly virulent against nymphs. The mortality %age significantly differed based on fungal isolates, stage of whitefly nymphs and conidial concentrations. Susceptibility of nymphs to fungal isolates decreased with advancement in age of nymphal instars. The highest mortality of young nymphs were observed by strains of *P. fumosoroseus* at a dose of 1×10^8 conidia/ml. The LC_{50} values of highly virulent strain were determined on several days after inoculation whereas LT_{50} values at different doses. The results indicated that *P. fumosoroseus* strains have potential as microbial control agent in Pakistan.

EFFECT OF HOT AND COLD WATER EXTRACTS OF TOBACCO, NEEM AND GARLIC ON THE MORTALITY OF *HETEROTERMES* SPP

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Different concentrations *i.e.* 5%, 10% and 20 % of tobacco, neem and garlic extract in hot and cold water were tested to compare the toxicity effect against termite specie *Heterotermes* at NIFA, Peshawar. The 20% solution in cold and hot water extract of garlic and tobacco caused 100 % mortality on 2nd day whereas the same concentration of neem didn't cause such mortality even after 10 days. Whereas the 10% cold water extract solution of garlic and tobacco caused 100% mortality after 6 and 8 days respectively. While the neem extract in both treatments at all the concentrations was ineffective even after 10 days. The 5% solution of tobacco in cold water caused 100 % mortality after 10 days and in hot water it caused only 77% mortality. So, tobacco and garlic at 10% solution concentration were effective against termite.

SOME STUDIES OF FOOD AND FEEDING BEHAVIOR IN FOUR SPECIES OF FIDDLER CRAB FOUND ALONG THE COAST OF PAKISTAN

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The food and feeding habits of four species of fiddler crabs belonging commonly inhabiting in Sandspit and Korangi creek mangrove areas of Pakistan, were analyzed. The spoon tipped setae were identified as the filter organ for the food and help in ingestion of

food particles. Food searching or feeding direction was found to be species specific. The feeding ground was positively correlated with the crabs burrow diameter. The stomach fullness and the composition (sediment and food items ratio) of the food ingested were recorded. Results shows that the percent mud contents were high in *Uca urvellie* and *Uca sindensis* as these two species are found in fine sediment as compare to *Uca annulipes* and *Uca chlorophthalmus*. *Uca urvellie* was observed also as the predator species, other species of fiddler crabs were observed as the omnivorous with some food items preference.

POPULATION STATUS OF RUFOUS-VENTED PRINIA (*PRINIA BURNESII*) IN PAKISTAN

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Random Amplified Polymorphic DNA (RAPD) technique was used to assess the population genetics of Rufous-vented Prinia *Prinia burnesii*, for that genetic material was obtained from three distant sites along western bank of River Indus. These sites include Chashma barrage (32°50'N, 71°20'E), in the north; Taunsa barrage (30°45'N, 70°45'E) and the southern population from Guddu barrage (28°26'N, 69°44'E). A sum of 14 positive primers were used to check the genetic variability between three populations of the species. The results indicate that they are not only geographically isolated but genetically as well. The genetic distance between Populations ranged from 0.149 to 0.2652. The Polymorphism levels were recorded 48% to 83% within the populations and overall polymorphism was 99.46%.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF *TURNICIDAE*, *GRUIDAE* AND *OTIDIDAE* IN PAKISTAN

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It has been observed that Pakistan lacks complete and updated taxonomic data on its avifauna with particular reference to subspecies level. Hence, an attempt has been made to make a review of taxonomical citation of class Avies, order Gruiformes including *Turnicidae*, *Gruidae* and *Otididae*. It was revealed from the study that *Turnicidae* family includes a genus *Turnix* that represents four species *T.sylvatica*, *T.tanki*, *T.blanfordi* and *T.suscitator* with three subspecies *T.s.dussumier*, *T.s.taigoor*,

T.s.taijoor and *T.t.tanki*. However *Gruidae* family including two genera *Grus* and *Anthropoides*. The genus *Grus* represents five species *G.nigricolis*, *G.antigone*, *G.cinerea*, *G.leucogeranus* and *G.grus* with including *G.g.lilfordi* and two nominate races, i.e., *G.a.antigone* and *G.l.leucogeranus*. However genus *Anthropoides* represents *A.virgo* with a nominate race *A.v.virgo*. Additionally, *Otididae* family represents seven genera, *Ardeotis* representing single species as *A.nigriceps* while, *Houbaropsis* represents another species *H.belgalensis* and *Houbara* representing *H.macqueeni* as sole species. However *Sapheotides* represents only species as *S.indica*, whereas, *Tetrax* represents *T.tetrax* its only race as *T.t.orientalis* from Pakistan. However, genus *Otis* represents two species *O.tarda* and *O.undulata* with three subspecies *O.t.dybowskii*, *O.u.macqueenii* and *O.u.jacquin*. Nevertheless, *Chlamydotis* represents single species as *C.undulata* with the only representative called *C.u.macqueenii* from the country. The study concludes the status of above mentioned three families represent altogether eighteen species, nine subspecies with four nominated species from Pakistan. Out of these seven are resident, two are breeder, three are summer visitor, five are winter visitor, two are passage migrant and one is vagrant.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF GAVIIDAE, PODICIPEDIDAE, PROCELLARIIDAE, HYDROBATIDAE AND PELECANIDAE IN PAKISTAN

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It has been observed that the avifauna has not been given proper attention with particular reference to the subspecies level. Thereby, an attempt has been extended to review the taxonomical status of the class Aves. The order *Gaviiformes*, family *Gaviidae*, genus *Gavia* which viz., only species *Gavia stellata* with subspecies *G.s.stellata* representing from Pakistan. However, in order *Podicipediformes*, family *Podicipedidae* includes two genera, which are *Tachybaptus*, *Podiceps* which represents five species *T.ruficollis*, *P.cristatus*, *P.griseogen*, *P.auritus* and *P.nigricollis* with four subspecies *T.r.ruficollis*, *P.c.cristatus*, *P.g.griseogen* and *P.n.nigricollis*. Secondly, order *Procellariiformes*, have two families *Procellariidae* and *Hydrobatidae*, where family *Procellariidae* contains two genera *Puffinus* and *Bulweria*. These two genera have four species *P.tenuirostris*, *P.iherminieri*, *P.pacificus* and *B.fallax* with three subspecies *P.t.tenuirostris*, *P.i.iherminieri*, and *P.p.pacificus*. Thirdly, family *Hydrobatidae* representing genus *Oceanites* has one species *O.oceanicus* with subspecies *O.o.oceanicus* from Pakistan. Whereas, order *Pelecaniformes*, contains five families *Pelecanidae*, *Phalacrocoracidae*, *Anhingidae*, *Sulidae* and *Phaethontidae*. Out of which, one family *Pelecanidae*, with genus *Pelecanus*, representing three species *P.onocrotalus*, *P.philippensis*, *P.crispus* alongwith three subspecies *P.o.onocrotalus*, *P.p.philippensis*,

P.c.crispus from Pakistan. The study revealed that the above families represent fourteen species overall, with twelve subspecies, where, three are vagrant, two breeders, two passage migrant, three winter visitors, two summer visitors and two resident in Pakistan.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF *PANDIONIDAE* AND *FALCONIDAE* IN PAKISTAN

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It has been observed that the bird-fauna of Pakistan has not been given proper attention, with particular reference to its taxonomic status. Thereby, an attempt has been extended to review the taxonomic status of families *Pandionidae* and *Falconidae* to their trinomial level. According to the study, family *Pandionidae* represents the only genus *pandion* with the only nominated species *P. haliaetus* and family *Falconidae* includes the only genus *Falco* having eleven species with eighteen subspecies in total from Pakistan. These include *F. peregrinus calidus*, *F. p. peregrinator*, *F. p. babylonicus*, *F. jugger*, *F. cherrug cherrug*, *F. c. milvipes*, *F. concolor*, *F. subbuteo subbuteo*, *F. s. centralasiae*, *F. columbarius insignis*, *F. c. pallidus*, *F. c. hristiani-ludovici*, *F. tinnunculus tinnunculus*, *F. t. interstinctus*, *F. naumanni pekinensis*, *F. chicquera*, *F. severus rufipedoides* and *F. amurensis*. Out of these eighteen subspecies of *Falconidae* three are summer visitor, four residents, six winterers, one vagrant, three breeders and one passage migrant, whereas, the only species of *Pandionidae*, *P. haliaetus* is winter visitor to Pakistan.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF *RALLIDAE* IN PAKISTAN

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It has been observed that the birdfauna of Pakistan has not been given proper attention, with particular reference to its taxonomic status. Thereby, an attempt has been extended to review the taxonomic status of family *Rallidae* upto trinomial level. The study revealed that family *Rallidae* represents nine genera, thirteen species with fifteen subspecies in Pakistan. Out of these, five are winter visitor, four summer visitor, four residents and two vagrants to Pakistan. Nine genera included in the family *Rallidae* are *Rallus*, *Crex*, *porzana*, *Rallina*, *Amaurornis*, *Gallinula*, *Gallicrex*, *Porphyrio* and *Fulica*. The genus *Rallus* represents the only species *R. aquaticus* with two subspecies *R. a. korejewi* and *R. a. indicus*. Whereas, genus *Crex* represents one species *C. crex* with only

subspecies *C. c. crex*. But genus *Porzana* includes five species i.e. *P. fuscus*, *P. akool*, *P. pusilla*, *P. porzana* and *P. parva* with five subspecies viz; *P. f. fuscus*, *P. a. akool*, *P. p. pusilla*, *P. p. porzana* and *P. p. parva*. Whereas, genus *Rallina* includes only species *R. eurizonoides* with subspecies *R. e. amauroptera*. Genus *Amaurornis* consists of one species *A. phoenicurus* with only subspecies *A. p. chinesis*. Genus *Gallinula* represents the only species *G. chloropus* with one subspecies *G. c. indica*. Genus *Gallicrex* includes the only species *G. cinerea* with one subspecies *G. c. cinerea*. However, genus *Porphyrio* represents the only species *P. porphyrio* with two subspecies *P. p. seistanicus* and *P. p. poliocephalus* and genus *Fulica* contains the only species *F. atra* with one subspecies *F. a. atra*.

**GENETIC VARIABILITY IN BLACK PARTRIDGE (*FRANCOLINUS*
FRANCOLINUS, GALLIFORMES) POPULATIONS IN PAKISTAN**

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Despite their high commercial value, there is little or almost no information available on the genetic structure of *Francolinus francolinus* in Pakistan. This relatively sedentary gallinaceous species has differentiated geographically as well as ecological features and morphology. The development of molecular techniques has led to a great increase in our knowledge of population genetics. So, Random Amplified Polymorphic DNA (RAPD) markers were used to characterize genetic heterogeneity within and among five populations of *Francolinus francolinus*. The study revealed that RAPD markers were effective in detecting polymorphism in the studied species. Twenty three individuals from five geographically distinct populations named as Alipur, Baitsuvai, Chakwal, Haroonabad and Rakni were sampled. A total of 269 different RAPD bands were generated using fifteen primers. Estimates of the percentage of overall polymorphism (87.79%), Shannon's diversity information index (0.421 ± 0.22) and Nei's gene diversity index (0.276 ± 0.17) were comparatively high in the five populations, high levels of genetic variation were revealed at the population level ($G_{ST} = 0.430$) and the populations of Rakni and Chakwal was found to have the highest genetic diversity among all populations and strong genetic differentiation. Genetic distance (F_{ST}) ranged from 0.2709 to 0.4811 and the differentiation between pair-wise populations was significant when individual pairs of populations were compared. Gene flow (N_m) estimated, was found to vary from 0.729 to 2.178 between pair-wise populations. The significant proportion estimated for G test ranged from 22.22% to 42.1%. The F_{ST} values found for *Francolinus francolinus* indicate that the populations of those two species are genetically structured that conformed to a strong isolation by distance relationship among the populations. A significant relationship between genetic distance (pairwise F_{ST}) and

geographic distance was observed. Of special interest was the fact that genetic differentiation of the five populations studied was more between the populations than within a population. The loss of genetic variability in populations was attributed to inbreeding, limited gene flow due to sedentary nature, fragmentation of the once continuous range and subsequent genetic drift, may have contributed to shaping the population genetic structure of the species. Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendrogram based on Jaccard similarity indices was constructed. The five populations of the *Francolinus francolinus* can be subdivided into four clusters, cluster one comprised of two populations and remaining three populations form separate clusters. An understanding of population genetic structure is necessary for delineating appropriate management units. Therefore, these findings are important for a better understanding of the adaptive strategy of *Francolinus francolinus* and will be useful for conservation managers to work out an effective strategy to protect this important species at individual level in Pakistan.

PTILOCHRONOLOGICAL ANALYSIS UNDER FOOD AND NOISE STRESS ON BLUE ROCK PIGEON *COLUMBA LIVIA*

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In this study, the effect of food reduction on feather growth, the variation in growth rate of induced feathers among different conditions of food and the impact of noise stress on feathers along with nutritional stress was studied. Three groups were taken, one was the control group and the other two were the experimental group, all these groups had 10 pairs of wild pigeon *Columba livia*. nutritional and noise stresses were given to the birds kept in experimental group and were observed the effect of nutritional and noise stress on feathers where as the birds kept in control group was remained in controlled conditions and ample food was given to them. Ample food was given to control flock, average food with noise stress to noise flock and the three percentages of average food (20% less than average, 40% less than average and 50% less than average) to food stress flock in three different intervals. For noise stress Telinga microphone was used to record the noise level of urban areas and then played back this noise at 700 Hz with the help of sound system in intervals. Induced molting was performed in control and noise groups just for one time in two sequences but in food stress group induced molting was performed for three times in two sequences under different food levels to compare the occurrence and intensity of impact of three levels. This study showed that the number of fault bar per flock was 70 at 20% less food, the number of fault bar per flock was 86 at 40% less food and the number of fault bar per flock was 101 at 50% less food. It also concluded that the fault bar widths were negatively correlated with food levels with $P = 0.051$. Tail lengths variations at three food levels was explained which clearly showed positive correlation between food reduction and tail length with $P = 0.13$. The weight of

individual and induced feather mass were positively correlated with food level. The number of fault bar, width of fault bar, tail length, induced masses in food stress flock were greater as compared to control which showed the impact of nutritional stress on feather growth. In the noise stress flock, the number of fault bar per flock was 63. In control flock, due to captivity fault bars were also observed in the control flock. The number of fault bar per flock was 55. This study showed that the difference between the fault bar width in primaries of noise stress flock and control flock was statistically significant at $P = 0.011$. The difference between fault bars widths of rectrices of these two groups was statistically insignificant at $P = 0.760$ and the difference between tail lengths of these two groups was statistically significant at $P = 0.001$. The number of fault bar, width of fault bar, tail length, induced masses in rectrices were greater as compared to primaries of all flocks which proved "the fault bar allocation hypothesis". The number of fault bar, width of fault bar and tail length in noise flock were also greater as compared to control which showed the impact of noise stress on feather growth but food was also involved there. So, it was shown experimentally that that fault bars resulted from malnutrition and were therefore act as a record of the bird's nutritional status while growing or molting.

**GENETIC DIVERSITY OF DIFFERENT POPULATIONS OF BANK MYNA
*ACRIDOTHERES GINGINIANUS***

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Random amplified polymorphic DNA (RAPD) markers were used to analyze the genetic diversity, genetic structure as well as genetic distance with in and among four different populations of Bank Myna (*Acridotheres ginginianus*). To achieve this goal 16 *Acridotheres ginginianus* were captured from four different sites of Punjab. These sites included D.G. Khan 30°03' N, 70°38' E, Khanewal 30°18'0 N, 71°56'0 E, Jahanian 30°18'0"N 71°56'0" E, Gujranwala 32.16° N, 74.18° E. Here we used 10 positive RAPD primers to generate the data which indicated 191 polymorphic bands with average interpopulation polymorphism of 81.11% (overall loci), ranged from $P=11.25\%$, (Jahanian population) to $P=49.74\%$ (Gujranwala population). Nie's genetic diversity measured by this study was very high $H=0.318$ and $S=0.467$ (overall). The genetic diversity at population level was variable showing range from $H=0.47(\pm 0.13)$, $S=0.0697(\pm 0.22)$ in Jahanian population to $H=0.195(\pm 0.20)$, $S=0.283(\pm 0.30)$ in D.G.Khan population. This study indicated that level of genetic diversity at species as well as population level was higher than average. The G-test polymorphic loci were estimated ranged from 21.31% to 39.47%. Gst values ranged from 0.34 to 0.53. This assumption is also supported by the number of migrants among the populations. The minimum numbers of migrants per

generation ($N_m=0.44$) was observed between Jahanian and Khanewal populations. Whereas, maximum number of migrants per generation ($N_m=0.94$) was estimated between populations of D.G.Khan and Gujranwala. The Nei's genetic distance ranged from (0.243) D.G.Khan and Jahanian to $D=0.341$ Gujranwala and Jahanian populations. Fisher exact test reported the high differentiation among the populations. In this study the highest differentiation is observed between Jahanian and Gujranwala ($\chi^2 P=0.5926$, $\chi^2=8.296$). Genetic similarity dendrogram assigned *Acridotheres ginginianus* into two clusters, the D.G.Khan, Jahanian and Khanewal grouped into cluster I, whereas, the Gujranwala population forms a separate cluster II. The contrary Principal Coordinate analysis showed that majority of birds belonging to same population, are grouped together in cluster formation. Most of the populations were clearly in isolation. Only birds from the Jahanian population seem to be genetically overlapping with the D.G.Khan population. From our findings, we concluded that ecological and biological factors and as well as the human impact on habitat has contribute to the genetic structure of observed species. In this respect, RAPD markers may provide the most useful tool for better understanding the *A. ginginianus* genetic variation and gene flow. The RAPD data analysis enables us to pioneer in estimation of genetic diversity of *A. ginginianus* at the local level.

POPULATION GENETIC ANALYSIS OF COMMON MYNA *ACRIDOTHERES TRITIS*

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Random amplified polymorphic DNA (RAPD) markers were used to analyze the genetic variation within and among four populations in the Punjab. Percentage of polymorphic bands, Nei's gene diversity index and Shannon's diversity information index were estimated to be very high with in the four populations ranging from $H=0.0984$, $S=0.1445$ (Gujranwala) to $H=0.2117$, $S=0.3034$ (Dera Gazi Khan). Theta P test shows that there are genetically significant differences in all populations statistically ranging from 0.444 (Azad Kashmir) to 0.765 (Dera Gazi Khan). G_{ST} results also indicated that at species level very high differentiation is present among populations with 0.580. Population genetic distances between all pair wise populations range from $D=.2749$ (Dera Gazi Khan and Khanewaal) to $D=0.5471$ (Gujranwala and Khanewaal). Dendrogram of populations clearly form three clusters, cluster one comprises of population of Jehlum valley (Azad Kashmir), second cluster consists of populations of Khanewaal and Dera Gazi Khan and third cluster consists of population of Gujranwala. All these findings will help in future for the phylogenetic analysis of this species.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF *SCOLOPACIDAE* IN PAKISTAN

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Pakistan lacks complete and updated taxonomic data of avifauna with particular reference to the subspecies level. Therefore, an attempt has been extended to review the taxonomic status of the class Aves, order *Charadriiformes*, family *Scolopacidae* up to trinomial level. This family includes sandpipers, curlews, godwits, shanks, woodcocks, stints, phalaropes, tattlers, snipes and dowitchers. The study revealed eighty nine species worldwide and thirty one species from Pakistan. These are vagrant, winter visitors, summer visitors, passage migrant, resident and breeders to Pakistan. The family *Scolopacidae* consists of six subfamilies including *Arenariinae*, *Tringinae*, *Scolopacinae*, *Gallinagicinae*, *Phalaropodinae* and *Calidrinae*. The subfamily *Arenariinae* includes only genera, *Arenaria*, which represents species *Arenaria interpres* and subspecies *A.i.interpres* from the country. The subfamily *Tringinae* further includes five genera, which are *Numenius*, *Xenus*, *Limosa*, *Actitis* and *Tringa*. These genera altogether consist of twelve species and nine subspecies from the country. However, the subfamily *Scolopacinae* includes only genera *Scolopax*, which represents species *Scolopax rusticola*. The subfamily *Phalaropodinae* includes one genera *i.e.*, *Phalaropus*, which represents two species *viz.*, *Phalaropus lobatus* and *Phalaropus fulicarius* from the country. While the subfamily *Gallinaginae* includes two genera *Lymnocyptes* and *Gallinago*. These genera include five species, Whereas, the subfamily *Calidrinae* includes three genera that are *Calidris*, *Limicola* and *Philomachus*, having ten species and one subspecies in total.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF *MOTACILLIDAE* IN PAKISTAN

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Bird is an emperor of the sky better to become a legend of the land. Class Aves is the most diverse class of terrestrial vertebrates. However in Pakistan this class has not been studied with proper attention with particular reference to its taxonomic status. Therefore, an attempt has been made to review the taxonomic citations of class Aves, Family *Motacillidae* upto their subspecific level in Pakistan's boundary. The present work is based entirely on a careful review of literature on Order *Passeriformes*, Family

Motacillidae upto trinomial status having three genera named as *Anthus*, *Motacilla*, and *Dendronanthus*. These genera have sixteen species with twenty three subspecies from Pakistan where one is vagrant, nine common, two rare passage migrant, one scarce, one straggler and two winter migrant. The Genus *Anthus* includes ten species named *A.campestris*, *A.cervinus*, *A.hodgsoni*, *A.novaeseelandiae*, *A.pratensis*, *A.roseatus*, *A.similis*, *A.spinoletta*, *A.sylvanus* and *A.trivialis* with twelve subspecies *A.c. campestris*, *A.h. hodgsoni*, *A. n. rufulus*, *A. n. waetei*, *A.n. richardll*, *A.p.pratensis*, *A.s.decaptus*, *A.s.jerdoni*, *A.s.japonicus*, *A.s.scutelli*, *A.t.trivialis* and *A.t. haringtoni*, where the genus *Motacilla* includes five species known as *M.alba*, *M.cinerea*, *M.citreola*, *M.flava* and *M.maderaspatensis* with a total of eleven subspecies *M.a. alboides*, *M.a. dukhunensis*, *M.a. personata*, *M.c. caspica*, *M.c. calcarata*, *M.c. citreola*, *M.c. werae*, *M.f. beema*, *M. f. leucocephala*, *M.f. melanogrisea*, *M.f. thunbergi* and the genus *Dendronanthus* having the only species known as *D.indicus* with only a nominate subspecies *D.i.indicus*.

**AN AVIAN SYSTEMATIC REVIEW AND STATUS OF
PHALACROCORACIDAE, ANHINGIDAE, SULIDAE, PHAETHONTIDAE,
PHOENICOPTERIFORMES IN PAKISTAN**

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It has been observed that Pakistan's bird fauna has not been given proper attention with particular reference to its taxonomic status. Thereby, an attempt has been extended to review the taxonomic status of different families at their trinomial level. Order *Pelecaniformes*, includes five families *Pelecanidae*, *Phalacrocoracidae*, *Anhingidae*, *Sulidae*, *Phaethontidae*, *Phoenicopteriformes*. Family *Phalacrocoracidae* includes only genus *Phalacrocorax* having four species, *P.carbo*, *P.fuscicollis*, *P.niger*, *P.pygmeus* and three subspecies *P.c.carbo*, *P.f.fuscicollis*, *P.n.niger* from Pakistan. Family *Anhingidae* includes genus *Anhinga* having only species *A.melanogaster* and subspecies *A.m.melanogaster* from the country. Family *Sulidae* includes genus *Sula* with only species *S.dactylatra* and subspecies *S.d.melanops* from Pakistan. Family *Phaethontidae* includes genus *Phaethon* having only species *P.aethereus*. Order *Phoenicopteriformes*, Family *Phoenicopteridae* includes only genus *Phoenicopus* having two species *P.ruber*, *P.minor* and two subspecies *P.r.roseus*, *P.m.minor*. The study revealed that all these families represents nine species and seven subspecies, where, one is vagrant, four residents, one passage migrant, four breeders, four visitors, and one winter visitor in Pakistan.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF *TIMALIIDAE* AND *HYPOCOLIIDAE* IN PAKISTAN

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Pakistan lacks complete and updated taxonomic data about avifauna, with particular reference to the sub-species level. Therefore, a study aimed at reviewing systematics and taxonomical citation of class *Aves*, order *Passeriformes*, family *Timaliidae* and family *Hypocoliidae* were devised. These include, Babblers, Sibias, Laughing thrushes, Shrike Babblers and Grey Hypocolius or Shrike bulbul. The study revealed that the family *Timaliidae* represents 11 genera in Pakistan, namely *Pomatorhinus*, *Stachyris*, *Chrysomma*, *Turdodius*, *Garrulax*, *Trochalopetron*, *Ianthocincla*, *Leiothrix*, *Peteruthius*, *Heterophasia* and *Panurus*. These genera represent 18 species and 5 sub-species in Pakistan, namely *P.erythrogeus*, *S.pyrrhops*, *C.sinense*, *C.altirostre*, *T.striata*, *T.earlei*, *T.malcolmi*, *T.caudata*, *G.albogularis*, *T.erythrocephalus*, *T.variegatus*, *T.lineatus*, *I.rufogularis*, *L.lutea*, *P.flaviscapitis*, *P.xanthochlorus*, *H.capistrata*, *P.biarmicus*, *T.caudatus caudatus*, *T.caudatus huttoni*, *T.lineatus lineatus*, *T.lineatus gilgit* and *T.lineatus balkvitchi*. Whereas, in family *Timaliidae* one species is vagrant, other is rare, one is extinct (last reported in 1873) and 15 species are resident to Pakistan. The *Hypocoliidae* family represents a genus *Hypocolius* with only species *Hypocolius ampelinus* in Pakistan. This is passage migrant and winter visitor to Pakistan.

AN AVIAN SYSTEMATIC REVIEW AND STATUS OF *COLUMBIDAE* IN PAKISTAN

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Pakistan lacks complete and updated taxonomic data of avifauna which has not been given proper attention, with particular reference to the subspecific level. Therefore, a study was devised to ascertain and review systematic taxonomical citation of class *Aves*, order *Columbiformes*, family *Columbidae*. The study revealed that the order *Columbiformes*, family *Columbidae*, represents five genera in Pakistan. These are *Columba*, *Streptopelia*, *Chalcophaps*, *Treron*, *Macropygia*, which represent nineteen species with seventeen subspecies in total. Out of these nineteen species, eight are resident, two breeders, one winter migrant, two vagrants, two rare and nomadic, two summer visitors and two winter visitors in Pakistan. Genus *Columba* contains six species including *Columba livia*, *Columba rupestris*, *Columba leuconota*, *Columba eversmanni*,

Columba hodgsonii, *Columba palumbus* with five subspecies in total, which are *C.l.neglecta*, *C.l.intermedia*, *C.r.turkestanica*, *C.l. leuconata*, *C.p.casiotis*. Whereas, genus *Streptopelia* includes six species, *Streptopelia turtur*, *Streptopelia orientalis*, *Streptopelia chinensis*, *Streptopelia senegalensis*, *Streptopelia decaocto*, *Streptopelia tranquebarica* with eight subspecies in total, *S.t. turtur*, *S.t.arenicola*, *S.o.meena*, *S.c.suratensis*, *S.s.cambayensis*, *S.s.ermanni*, *S.d. decaocto*, *S.t. tranquebarica*. Nevertheless, genus *Chalcophaps* includes the only species, which is *Chalcophaps indica*. However, genus *Treron* includes five species, *Treron bicinctus*, *Treron phoenicoptera*, *Treron sphenura*, *Treron pompadora*, *Terron phayrei* by representing three subspecies, *T.b.bicinctus*, *T.p.phoenicoptera*, *T.s.sphenura*. Finally, genus *Macropygia* represents only species, *Macropygia unchall* with subspecies *M.u.tusalia*.

**GENETIC STRUCTURE OF SEE-SEE PARTRIDGE (*AMMOPERDIX
GRISEOGULARIS*, GALLIFORMES) POPULATIONS FROM SUB-
HIMALAYAN MOUNTAIN RANGES OF PAKISTAN**

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We used Random Amplified Polymorphic DNA (RAPD) markers to investigate genetic structure of Two populations of see-see partridge (*Ammoperdix griseogularis*, Galliformes), an important game species of the Suleiman range, in the Pakistani Himalayan region. The percentage of polymorphic bands (94.05 %), Shannon's Information Index ($H = 0.4557$) and Nei's gene diversity ($I = 0.2984$) were comparatively high in this species. 17% of polymorphic loci were found to be having statistically significant difference in their allelic frequencies. G_{ST} (Nei's coefficient of genetic variation) values indicated moderately high level of differentiation ($G_{ST} = 0.08$). Genetic distance was $D = 0.0536$ and both populations are very divers and in isolation to some degree. Overall, our genetic data will support action plan aiming to preserve locally differentiated genetic resources that, in the future, could potentially result into ecologically and behaviorally differentiated populations. In the context of rapid environmental changes that Himalayas has been experiencing in the last decade, it seems worthy to maintain options for future adaptation

**DIVERSITY AND POPULATION ESTIMATIONS OF AVIAN FAUNA AT
PUNJAB FOREST RESEARCH INSTITUTE, GATWALA, FAISALABAD**

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Forest Research Institute (PFRI), Gatwala, Faisalabad, is situated 17 km away from Faisalabad towards Lahore. It is a complex of aquatic and terrestrial ecosystem. Present research was carried out during June to September; 2007. The area was weekly visited to study the diversity and population estimation of bird species. Binocular and zoom scope were used to spot and identify the bird species. The area was accommodating a large variety of birds. The avifauna was observed for aquatic and terrestrial habitats. Overall, sixty two bird species belonging to thirty two Families and twelve Orders were recorded during 4 months study period. According to the status of avian species, it was observed that out of 62 species, 17 species were migratory, 12 migratory and summer breeders, 5 winter visitors and 44 residents which were found throughout study period. Passeriformes was the most dominant order represented by 31 species belonging to 15 families, followed by Coraciiformes which was represented by 7 species belonged to 5 families. Piciformes and Charadriiformes were represented by 3 and 2 species with two families each, respectively. Order Accipitriformes, Ciconiiformes and Cuculiformes were represented by 4, 3, and 3 species belonging to single family each, respectively. Order Galiiformes and Columbiformes were represented by 2 species each belonging to single family. Order Gruiformes showed the presence of single species with single family. Maximum population of birds was observed during July and August, 2007 due to abundance of migratory and summer breeders.

**ACUTE TOXICITY OF IRON AND ZINC TO FISH AT VARIABLE
TEMPERATURE, pH AND HARDNESS OF WATER**

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Laboratory tests were conducted on 60-day fish viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* to determine the acute sensitivity (96-hr LC₅₀ and lethal toxicity) of iron and zinc at variable water temperature, pH and total hardness. The major modifying physico-chemical factors of metal's toxicity were water temperature, hardness and pH of water. All the three fish species showed variable sensitivity to iron and zinc at different sets of variables, i.e., temperature, pH and hardness of water. Among the three fish species, *Cirrhina mrigala* were significantly less sensitive to zinc at all sets of variable temperature, pH and hardness of water than that of *Labeo rohita* and *Catla catla*. However, *Labeo rohita* appeared as more sensitive to iron followed by that of *Cirrhina*

mrigala and *Catla catla*. At constant water hardness (100 mgL^{-1}) and low pH (7), the rise in water temperature from 25 to 30°C has resulted significant decrease in 96-hr LC_{50} of metals for all the three fish species. The mean 96-hr LC_{50} for fish decreased with decreasing total hardness and pH at 30°C . However, fish were less sensitive to metal toxicity at high pH (8) and hardness (200 mgL^{-1}). The sensitivity of fish to metal's toxicity increased with decreasing both the hardness and pH of water. However, toxicity of metals to the fish increased concomitantly with an increase in water temperature.

**FAUNAL DIVERSITY AND PREDATOR-PREY /PEST STATUS IN THE
SUGARCANE AND WEED CROP COMPLEX OF WHEAT IN FAISALABAD
DISTRICT**

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Faunal diversity is one of the significant cornerstones of agriculture which serves as sustainable agricultural products, pollinators, predators as well as Predator-prey/pest relationship performing a vital role in sustaining the prey/pest population. This study was aimed to investigate the faunal diversity and predator-prey/pest relationship in agroecosystem. The study was carried out in suburbs of Faisalabad on the farmer's fields. Data were collected from selected quadrates of 1 meter square in both crops on weekly basis by using sweep net, hand picking, were analyzed statistically. A total of 2804 and 1428 specimens belonging to different orders were captured from weed-crop complexes of wheat and sugarcane crops respectively. As many as 72, 82 species from both crops respectively collected of which 35 and 46 were predators including insects and spiders. Other arthropods were 35, 35 playing prominent role in limiting the prey/pest population. Whereas, two species were decomposers playing a key role in nutrient recycling captured from both weed-crop complexes of wheat and sugarcane respectively. Such data on trophic status has very little documentation. This study will lead to targeted work on biology and ecology of each species in the agroecosystem.

**EVALUATION OF GROWTH PERFORMANCE OF MAJOR CARPS UNDER
DIFFERENT PRIMARY PRODUCTIVITY LEVELS**

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The present project was planned to evaluate the growth performance of major carps viz, *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* under different primary

productivity levels which was determined by light penetration. The experiment was conducted for six months using three earthen ponds. Each pond was stocked with 20 *Labeo rohita*, 15 *Catla catla*, and 15 *Cirrhinus mrigala*. Light penetration *i.e.* 10cm, 20cm and 30cm of three ponds water was maintained in pond-1, pond-2 and pond-3, respectively by adding appropriate amount of cowdung. At the end of the experiment in pond-1 the average body weight gains of *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* was found to be 395.8 ± 7.52 , 398.6 ± 6.72 and 323.4 ± 7.35 g, respectively. In pond-2, the average body weight gains in *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* was 452.7 ± 4.35 , 461.7 ± 8.50 and 404.2 ± 6.53 g, respectively. While in pond-3, the average body weight gain was 372.0 ± 9.62 , 391.7 ± 9.32 and 311.9 ± 4.23 g for *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*, respectively. Pond-2 showed a marked increase in the fish production than pond-1 and pond-3. The net fish production of three fish species/ha/year was computed to be, 1531.4, 2031.8 and 1456.5 kg in ponds-1, 2 and 3, respectively.

ROLE OF DIFFERENT FISH FEED INGREDIENTS ON THE GROWTH PERFORMANCE OF MAJOR CARPS IN SEMI-INTENSIVE FISH CULTURE SYSTEM

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The present experiment was conducted to study the role of different fish feed ingredients on the growth performance of major carps in semi intensive fish culture system. Three earthen ponds located at Fisheries Research Farms, University of Agriculture, Faisalabad and designated as T_0 , T_1 and T_2 . Each pond was fertilized with cattle manure at the rate of 0.10g N/100g of wet fish body weight daily. The pond designated as control pond (T_0) was not supplied with supplementary feed while the pond designated as T_1 supplemented with sunflower meal and T_2 with corn gluten. The results suggested that the average weight gain of *Labeo rohita* was 331.5 ± 3.84 , 525.6 ± 3.58 and 467.9 ± 5.33 g while the average weight gain of *Cirrhinus mrigala* was 239.4 ± 6.68 , 411.9 ± 3.34 and 369.9 ± 3.33 in all the treatments (T_0 , T_1 and T_2) respectively. Net fish yield (both species together) was 1104.6, 2054.4 and 1794.85 Kg/ha/year under T_0 , T_1 and T_2 respectively. The gross fish production was calculated 1478.25, 2428.45 and 2169.85 kg/ha/year in T_0 , T_1 and T_2 ponds respectively. In all treatments, *Labeo rohita* showed the better growth performance followed by *Cirrhinus mrigala*. It also showed that by increasing level of crude protein (sunflower meal) in the diet of fish, there was a significant increase in fish yield. The body weight gained by major carps (*L.rohita* and *C.mrigala*) was maximum in ponds treated with supplementary feed (T_1 and T_2) as compared to control pond (T_0).

**GROWTH PERFORMANCE OF METALS MIXTURE STRESSED CATLA
CATLA IN SEMI-INTENSIVE POND CULTURE SYSTEM**

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Fingerlings of *Catla catla* were divided into two groups, one group was exposed to sub-lethal concentrations of metals mixture (Fe, Zn, Pb, Ni, Mn) while other was kept un-stressed as control in glass aquaria for 30 days. During stress period fish showed positive increment in weight as 1.01g while fork and total lengths also increased as 5.70 and 7.50 mm respectively. At the end of stress trial, both stressed and control fish were shifted to earthen ponds for study of growth parameters in semi-intensive culture for a period of six months. The ponds were fertilized with poultry droppings @ 0.16g nitrogen per 100g net fish weight daily. During each trial, fish was fed daily (6 days a week) with the feed of digestible energy equal to 2.90 Kcal.g⁻¹ and 35% digestible protein. Analysis of variance revealed that there exists statistically significant difference among months for average weight, total length and highly significant for fish fork length. Treatments showed statistically non-significant response towards weight increment but significant for fork length and total length. The interaction between months and treatments also remained statistically significant in terms of weight, fork length and total length increments ($p < 0.01$). On the basis of this study, it was concluded that stressed *Catla catla* under sub-lethal concentrations of metals mixture showed significantly lower values of weight, fork and total lengths than control fish when reared in semi-intensive pond culture system.

**ROLE OF SUNFLOWER MEAL ON THE GROWTH PERFORMANCE OF
MAJOR CARPS (*LABEO ROHITA* AND *CIRRHINUS MRIGALA*) IN SEMI-
INTENSIVE FISH CULTURE SYSTEM**

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Role of sunflower meal on the growth performance of major carps (*Labeo rohita* and *Cirrhinus mrigala*) was evaluated through a six-month grow-out trial in manured earthen ponds. *Labeo rohita* (50%) and *Cirrhinus mrigala* (50%) were stocked at combined density of 40 fishes/pond. The ponds were fertilized with cow dung at a rate of 0.10g N/100g of wet fish body weight daily while sunflower meal was provided as supplementary feed in T₁ at a rate of 2% of wet fish body weight daily and evaluated in

replicates. Provision of sunflower meal caused significantly higher increase in overall growth, SGR and net biomass yield of carps in T_1 as compared to control T_0 . Cow dung manure also well contributed in the growth of major carps. The net production in T_1 ($1648.0 \text{ kg ha}^{-1} \text{ 6 months}^{-1}$) was higher than those of control T_0 ($810.08 \text{ kg ha}^{-1} \text{ 6 months}^{-1}$). The study revealed the relative advantage of using sunflower meal along with cow dung manure in carp culture in semi-intensive systems.

GROWTH AND SURVIVAL RATE OF *CHANNA MARULIUS* UNDER THE INFLUENCE OF ARTIFICIAL FEED IN FERTILIZED PONDS.

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The project was planned to investigate the effect of supplementary feed on the growth performance of *Channa marulius* in fertilized ponds. Four earthen ponds were stocked @ 100 fish per pond and all ponds were fertilized with organic (cow dung) and inorganic (nitrophos) fertilizers @ 0.16 gN/100g of wet fish body weight daily and was added on weekly basis. The four ponds used in experiment were designated as T_1 , T_2 , T_3 and T_4 . The pond designated as T_1 was supplied with organic and inorganic fertilizers and kept as control pond, T_2 was supplied with fish meal, and T_3 was stocked with 30 tilapia as forage fish for *Channa marulius* and T_4 was supplied with supplemented feed of plant origin containing 30% C.P. Feeding was done @ 2% of wet fish body weight daily. The growth was measured in terms of body weight and total length. Water samples from the fish pond was obtained on fortnight basis and analyzed for physico-chemical characteristics. The maximum weight gain was observed as (27.4 g) in T_2 as compared to the T_1 (18.6 g), T_3 (25.0 g) and T_4 (17.0 g). The water physico-chemical parameters were remained within the optimum limits. The survival rate was observed maximum in T_3 and growth was highest in T_2 .

EVALUATION OF GROWTH AND PRODUCTION OF SAUL (*CHANNA MARULIUS*) UNDER THE INFLUENCE OF ARTIFICIAL FEED AND TILAPIA MIXED CULTURE

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To evaluate the growth and production *Channa marulius* was stocked for six months in four earthen ponds designated as T_1 , T_2 , T_3 and T_4 , respectively @ 100 fish pond⁻¹. Ponds were fertilized with organic (cow dung) and inorganic fertilizer (

nitrophos) @ 0.16g N/100g of a wet fish body weight daily. T₁ was treated only with organic and inorganic fertilizer and was kept as control pond. T₂ was treated with fish meal. In T₃ tilapia was kept as forage fish. In T₄ fish meal and 30 tilapias were introduced. The growth was measured in terms of body weight and total length. The net fish production values were 74.94, 117.65, 233.32 and 430.92 kg/ha/year for T₁, T₂, T₃ and T₄, respectively. Mean values of water quality and fish growth parameters were made by using analysis of variance. Correlation coefficients were calculated among water quality parameters for each pond. Most of the ecological parameters such as dissolved oxygen, water temperature, and light penetration showed highly significant differences. The growth was highest in T₄ and minimum in T₁. It was due to the use of tilapias as a forage fish. However, some inter-pond differences were statistically non-significant.

WINTER GROWTH OF CARPS UNDER THE INFLUENCE OF INORGANIC FERTILIZER

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This project was planned to observe the influence of inorganic fertilizer on the growth of carps during low temperature. Two earthen ponds were selected and each pond was stocked with 44 fishes, *i.e.* Silver carp (*Hypophthalmichthys molitrix*), Rohu (*Labeo rohita*) and Mori (*Cirrhinus mrigala*) at the ratio of 1:2:1 respectively. Ponds were treated with urea and poultry manure at the rate of 2% nitrogen/gm of wet fish body weight daily *i.e.* pond 1 urea and pond 2 urea + poultry. Fish growth parameters *viz*; body weight fork length and total length was recorded on weekly basis. Important water quality parameters were also recorded. Data thus obtained subjected to statistical analysis. Net fish production recorded in pond 1 was 495.06 kg/ha/year and in pond 2 was 791.08 kg/ha/year. Pond 2 showed 1.59 times more fish production as compared to pond 1. The fish showed relatively better growth during warmer months of study in both ponds.

EFFECT OF VARYING RATIO OF *CIRRHINA MRIGALA* AND *CATLA CATLA* AT CONSTANT STOCKING DENSITY IN CARP COMPOSITE CULTURE SYSTEM

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Fish *viz.* *Cirrhina mrigala*, *Labeo rohita*, *Catla catla*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* were stocked in the ratio of 1:2:1:1:1 and 1:1:2:1:1 in experimental ponds I and II, respectively. Phosphorus and Nitrogen were added in both

the ponds with the ratio of 1:1 by using Single Super Phosphate and NH_4NO_3 as phosphorus and nitrogen source, respectively, for a period of 30 weeks. Mean water quality values and fish growth parameters were made by using Factorial experiment. Correlation coefficients were calculated among water quality parameters for each pond. The ecological parameters such as water temperature, total hardness, magnesium, total solids, total dissolved solids and total alkalinity showed highly significant seasonal differences. However, non-significant difference was found between experimental ponds. Overall net production of fish in experimental pond I and II remained statistically non-significant with mean values as 2125.28 and 1913.08 kg/ha/year, respectively. The pond stocked with lower stocking ratio of *Catla catla* showed a little bit higher net production. The total production of *Catla catla* was less than *Cirrhina mrigala* in both the ponds. Overall Nitrogen Incorporation Efficiency (NIE) and Nitrogen Conversion Efficiency (NCE) were 3.61 and 0.1012 for pond I and 3.25 and 0.0910 for pond II, respectively.

LOSS OF DIVERSITY AND RELATIVE ABUNDANCE OF VARIOUS MACRO-INVERTEBRATE SPECIES IN THE HIGH INPUT CROP FIELDS OF SUGARCANE AND WHEAT

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Soil macro-invertebrates are an integral part of agricultural ecosystems. The modern and intensified agriculture has caused a significant reduction in soil macro-invertebrates resulting in negative impact on soil structure, hydrological processes, Gas exchange, detoxification and decomposition of organic matter. The present study was conducted to compare the diversity and relative abundance of invertebrates in wheat and sugarcane fields of high and low inputs of chemicals in district, Faisalabad. Soil samples were taken by core sampler from three microhabitats (open edge, sub-shadow and amidst the field) of each randomly selected field through two consecutive years. Soil macrofauna were hand sorted in the laboratory. A total 2744 specimens belonging to various orders and families was recorded from the two types of treatments as many as 1922 were from low input fields and only 822 specimens could be captured from high input fields. The abundance of macro-invertebrates in the low input fields was significantly higher than there of high input fields and species diversity in the three microhabitats of each field (open edge, Sub-shadow and amidst the field) was also higher in the low input of both sugarcane and wheat crops except amidst of wheat fields. Pulmonata, Isopoda, Hymenoptera, Haplotaxida and Coleoptera were the most abundant arthropod group in both the crops. The data showed that organically managed low input fields of both the crops supported well the abundance of arthropods including the predator, prey as well as recycler/ decomposers species.

ACUTE TOXICITY OF NICKEL TO *LABEO ROHITA* AND *CIRRHINA MRIGALA*

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Acute toxicity of nickel to fish viz. *Labeo rohita* and *Cirrhina mrigala* have been studied in terms of 96-hr LC₅₀ and lethal tests. The tests were performed, separately, at constant temperature (32°C), hardness (50 mgL⁻¹) and pH (7) of water. Physico-chemical variables viz. sodium, potassium, total ammonia, carbondioxide, dissolved oxygen magnesium and calcium were monitored on daily basis. These were found significantly differences between LC₅₀ and lethal concentrations of nickel for *Cirrhina mrigala* as 56.07 and 126.11 mgL⁻¹ and for *Labeo rohita* as 55.27 and 120.77 mgL⁻¹, respectively. *Labeo rohita* was most sensitive to nickel than that of *Cirrhina mrigala*. The metal concentration of test mediums showed positively significant correlation with carbondioxide, total ammonia, potassium, sodium and electrical conductivity. However, the showed inverse relationship with dissolved oxygen for *Labeo rohita* and *Cirrhina mrigala*. The oxygen consumption by the fish decreased while ammonia secretion increased significantly with the increase in the metal concentration.

STATUS OF HERBIVORE-CARNIVORE COMPLEX IN *BRASSICA* CROP FIELDS

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Biological diversity within ecosystems, including agro-ecosystems, provides a range of biological functions and there are some indications that more diverse agricultural systems may enhance natural control of pests. The family *Brassicaceae* includes a number of species that have considerable nutritional and economic values. The present study has met the objective of exploring and evaluating arthropod population interactions and suspected synergies for the crop plants in terms of pest reductions. Data was collected with the help of hand net and by hand picking from selected quadrates of 1 m². A total of 9067 specimens, 8946 from the crop plants and 121 from the weed plants that were found associated to crop, belonging to 81 species were sampled from *Brassica*-weed complex, of which 1321 predator, 5896 pests/prey and 1729 were found to be omnivores. Cruciferous crops were highly vulnerable to pests like many aphids and some lepidopteran species. Maximum predator populations were observed during March and April because they develop well at 22-25 °C and due to the presence of their preferred prey. Predators dominated the weed plants while pests/prey populations dominated the

crop plants throughout the cropping season. Therefore the study evidenced the synergetic effects of phyto-morphic diversity helping pests control on Crucifers.

FEEDING LINKS OF VARIOUS ARTHROPOD POPULATIONS OCCURRING ON VEGETABLES

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Vegetable cropping systems are plagued by a complex of foliar and soil dwelling insect pests that significantly reduce the quality and quantity of harvested crop. Agro-ecosystem disturbances such as tillage, pesticide application, field sanitation and cropping patterns create environments in which herbivores and natural enemies are unable to keep their populations in natural balance and pest infestations overwhelm reducing quality and quantity of production. Present study was aimed at sorting out the frequency of beneficial organisms and their feeding links with the other biota of the vegetable field systems in district Faisalabad. Main vegetable crops sampled were, Bitterguard, Pumpkin, Garlic, Raddish, and Carrot along with some other crops. Data was collected with the help of hand net and by hand picking from selected quadrates of 1 m². During this study period, 3055 specimens were sampled from crop-weed complex, containing 95 species, of them 2906 were from crop plants and 149 from weed plants, among them, 47 pests/prey, 42 predator and 6 omnivorous species were reported. As many as 667, 525, 275, 274, 131 specimens were sampled from Garlic, Bitterguard, Pumpkin, Raddish, and Carrot respectively and 848 from other vegetable crops along with 335 Pulmonates. The most prevalent form of disturbance in vegetable production was pesticide application which was deleterious to herbivores and their natural enemy populations, as well as herbivores on co-existing non-crop plants. Predator populations dominated large scale cultivated vegetable crops while in small scale vegetable crops pests/prey populations pre-dominated probably due to the low application of pesticides.

STATUS OF WEEDS HARBORING FAUNA IN SUGARCANE, FODDER, WHEAT AND BRASSICA CROPS

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Weeds are considered as constraints on crop production, yet their presence in the crop fields increases phytomorphic heterogeneity for the subsistence of diverse arthropod fauna including predators, preys and pests. Fields of sugarcane, fodder, wheat and

brassica were randomly selected for collection of arthropod fauna in and around the weed plants during an entire season of the crop in the fields. 29 species of different weeds were explored from the selected crops with majority of species present in Sugarcane crop. The sampling period extended from July 2008 through June 2009. Quadrature method was used for sampling. A total of 5575 specimens belonging to 8 orders and 33 species of arthropods were sampled from different weed plants. Most of the weeds were present along the periphery and 2-3 feet inside the cropfield bearing different faunal species. The predator-prey ratio for weed plants was about 1: 3. Thus it is of indication that severe perturbation in their trophic structure probably due to frequent use of herbicides, pesticides and chemicals on the crops like wheat, fodder and brassica.

TROPHIC STATUS OF FODDER CROPS IN DISTRICT FAISALABAD

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Crops and its related diversity is the intrinsic and important part of the ecosystem. Fodder fauna performs better role in sustaining nearby crops, such as sugarcane and wheat etc. Present study aimed to investigate the trophic status of fodder crops in district Faisalabad, using quadrature method fauna in the randomly selected fields of fodder-weed complex. Fodder crops sampled were *Trifolium* spp. Maize and *Brassica*. A total of 2126 specimens belonging to 91 species were collected from all the fodder crops. Of which, 21 species were common while 39, 21 and 10 species represented by 1530, 403 and 193 specimens were sampled from *Trifolium* spp. Maize and *Brassica* fields and weeds therein. It was observed that less or no use of chemicals enhanced the diversity and number of predator species, where some pest populations were observed in higher number in chemically treated fields of fodder-weed complex. Weeds harbored high abundance of predators and low pest populations. Of crop plants, Maize showed abundant predators, *Brassica* and *Trifolium* spp. showed dominant predator's numbers and higher number of pest species. Thus by introducing or using useful phyto-morphic heterogeneity in the fodder crop system, pest outbreak can be avoided.

EFFECT OF SPECIES COMBINATION AND THEIR DENSITY RATIO ON GROWTH PERFORMANCE AND WATER QUALITY IN CARP POLYCULTURE SYSTEM

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An experiment was conducted to determine the effect of interaction between species and their density ratio on growth performance and water quality in carp

polyculture system. Fingerlings of three carps viz; *Cirrhinus mrigala*, *Ctenopharyndon idella* and *Hypophthalmichthys molitrix* were stocked in the ratio of 15:45:45 and 15:45:30 in experimental pond1 and 2, respectively. Analysis of variance on wet weight, fork and total length of three fish species showed that the fortnights, species combination and ratios exerted significant effect ($P < 0.01$) on growth. Among three fish species in pond1, grass carp gained significantly higher body weight, followed by mrigal and silver carp (322, 282 and 260g) while, in pond2, silver carp gained higher body weight followed by mrigal and grass carp (400, 299 and 284g), this may be due to difference in stocking ratios. Results showed that, pond 2 stocked with lower ratio of silver carp produce higher production of mrigal which may be due to competition in feeding while, pond 1 stocked with higher ratio of silver carp produce higher production of grass carp. Overall net fish production of pond 1 (4036.52 kg) and pond 2 (4207.88kg) statistically varied non significant. Among ponds there was no significant difference in gross fish production y^{-1} (5148.34 and 5112.32 kg) and net fish production y^{-1} (4036.52 and 4207.88 kg). Most of the ecological parameters such as temperature, light penetration, dissolve oxygen, pH, alkalinity and total hardness showed highly significant seasonal differences ($P < 0.01$) but, remained favorable during whole period of study and contributed to increase the planktonic biomass.

FEEDING HABITS OF COMMON QUAIL (*COTURNIX COTURNIX*) IN RAWALPINDI, PAKISTAN

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This study was designed to investigate the feeding habits of common quail (*Coturnix coturnix*) in Rawalpindi, Pakistan. A total of 28 birds were hunted (12 male and 16 female) during September 2008 to May 2009 and gizzards were removed for feeding analysis. Out of 28 specimens two gizzards were empty and the remaining 26 specimens contained 84.30% seeds of plants viz; millet seeds (*Pennisetum Americanum*; 17.9%), sorghum seeds (*Sorghum bicolor*; 17.2%), chickpea leaves (*Cicer arietinum*; 10%), pohli seeds (*Carthamus oxyacantha*; 9.8%), swank (8.7%), baru seeds (*Sorghum halepense*; 6.6%), resin (4.2%) and sesame seeds (*Sesamum spp.*; 1%). The debris and stone contents in gizzards were comprised of 10.6 and 5.20%, respectively. It is concluded that main food items of common quail consisted of plant content in Rawalpindi, Pakistan.

ECOLOGY OF BLUE ROCK PIGEON (*COLUMBA LIVIA*) IN URBAN AREAS OF RAWALPINDI/ISLAMABAD, PAKISTAN

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This study was designed to study the ecology of blue rock pigeon (*Columba livia*) in the urban areas of Rawalpindi/Islamabad, Pakistan. Seasonal changes in population density, age group, sex ratio, roosting and nesting sites of blue rock pigeons were recorded in twin cities, Rawalpindi/Islamabad. In Islamabad, higher population density of pigeon was recorded in winter season followed by autumn, spring and summer season (13.82, 13.60, 10.70 and 9.3/Km², respectively). However, in Rawalpindi, the higher population density of blue rock pigeon was found in summer season followed by winter, spring and autumn (13.37, 11.88, 11.10 and 10.02 /Km² respectively). The male and female population of pigeon confirms 1:1 sex ratio during different seasons. However, adult and juvenile numbers of pigeon did not followed 2:1 ratio, adults being more than juveniles in all seasons. The roosting and nesting sites did not differ ($P > 0.05$) in different seasons in Rawalpindi/Islamabad. Highest population of pigeon was recorded in historical buildings (120), and lowest in parklands (1.25). It is concluded that population density of blue rock pigeon ranges 10-13/Km²; roosting and nesting sites remained similar in different seasons and type of buildings affect the blue rock pigeon population in Rawalpindi/ Islamabad, Pakistan.

BANDICOOT RAT (*BANDICOTA BENGALENSIS*): A NOVEL RESERVOIR OF PATHOGENIC BACTERIA AT POULTRY FARMS, RAWALPINDI/ISLAMABAD, PAKISTAN

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The prevalence of pathogenic bacteria was studied in fecal matter, urine and blood of bandicoot rat (*Bandicota bengalensis*) inhabiting poultry farms. For this purpose, fifteen bandicoot rats were captured with live traps from poultry farms of Rawalpindi/Islamabad, Pakistan and checked for presence of bacterial species. The fecal matter of bandicoot rat was found to be contaminated with *Escherichia coli* (7%),

Klebsiella spp. (27%), *Proteus* spp. (15%) and *Salmonella* spp. (20%). The *Proteus* spp (20%) and *Salmonella* spp. (7%) were also isolated from urine of bandicoot rat. This preliminary study showed that bandicoot rat serves as reservoir of bacterial infection of *Escherichia coli*, *Klebsiella* spp., *Proteus* spp. and *Salmonella* spp. at poultry farms.

SPATIAL AND TEMPORAL VARIABILITY OF COPEPODS FROM THE MANGROVE CREEK AREA ALONG THE KARACHI COAST

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Monthly zooplankton samples were collected from two stations of Korangi, Phitti mangrove creek area near the Karachi coast. Copepod formed the dominant taxonomic group in the zooplankton community. The total seventeen species of copepod pertaining to thirteen genera were identified. The spatial variations and diversity of copepods was compared as K-dominance curves. The K-dominance curves clearly indicated that there was difference in diversity of copepod species among seasons with highest density in Pre monsoon at both stations. *Acrocalanus longicornis*, *Paracalanus* sp. and *Acartia* sp. were observed as the dominating and abundantly found copepod species through out the study period.

REPRODUCTIVE BIOLOGY OF TWO PENAEID SHRIMP FROM THE SONMIANI BAY LAGOON BALOCHISTAN PAKISTAN

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Shrimp fishery plays a significant role in the economy of Pakistan. The vital aspects of reproductive biology, the spawning season and gonadal maturation is of special interest in fisheries management and is widely used as an indicator for minimum permissible capture size. The present study is designed with the aim to determine the maturation stages of ovaries and spawning seasons of *Penaeus indicus* and *Penaeus merguensis*, the commonly occurring penaeid shrimps in Sonmiani Bay Lagoon. Shrimps were collected from the local ice fisheries landing at Sonmiani Bay during July 2006 to June 2007. The procured shrimp samples were sorted, sexed and identified up to species. In *P. indicus* and *Penaeus merguensis*, five ovarian maturation stages were recognized by the variations in colour, as well as development and arrangements of cells.

These stages were designated as undeveloped, developing, nearly ripe, ripe and spent. Data indicate both species spawns throughout the year with 2 or 3 peaks, the peaks being different in different years. The peak spawning activity was observed in both species and during post and pre monsoon in *P. indicus* and North East and pre monsoon in *P. merguiensis*. The reproductive approach of penaeid showed iteroparity and they spawn frequently with a short interval between successive spawning and in a single year.

CRUSTACEAN DEVELOPMENTAL STAGES IN THE SEDIMENT SAMPLES AT TWO SITES OF PAKISTAN COAST

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The adults crustaceans, their larval stages and juveniles were screened from the sediment. Surprisingly there was no direct relations in number of adults with their larvae in the samples. The groups encountered were the cirripeds and malacostracans the later include tanaids, cumaceans, isopods and decapods. Sometimes no adult was present but its stages are observed as in the cases of palaemonid shrimp and penaeid shrimp. The presence of the larvae indicate the breeding seasons. The location of breeding grounds also may lie nearby as each sample had one or two individuals, possibly drifted to the sampling sites. Each larva in dissected, illustrated and identified to the lowest possible taxonomic level.

A NEW RECORD OF *CERAPUS OCEANICUS* LOWRY, 1985 (AMPHIPODA, ISCHYROCERIDAE) FROM PAKISTAN, NORTHERN ARABIAN SEA

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This paper reports on the new record of an Ischyrocerid amphipod genus *Cerapus* Say, 1817 and its species *C. oceanicus* Lowry, 1985 in the northern Arabian Sea. The family Ischyroceridae is also first time reported from Pakistani waters. This species (*C. oceanicus*) was previously reported only from Samoa Island, Fiji in the Pacific Ocean. The material collected from both the Sindh (Bulleji & NewPacha) & Makran (Taq) coasts is housed in the Marine Reference Collection & Resource Centre University of Karachi. The species is illustrated and described in the present paper.

ZONAL DISTRIBUTION AND DIVERSITY OF BRACHYURAN CRABS IN TWO MANGROVE AREAS OF SONMIANI, BALOCHISTAN

NOOR US SAHER AND NAUREEN AZIZ QURESHI

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The brachyuran crab diversity and densities were studied by transect analyses from four stations of two mangrove sites of Bhaira and Miani. Among all the macrofauna inhabiting in the mangrove swamps, brachyurans are among the most important taxa with regard to species diversity and total biomass. The brachyuran crabs were collected from the low tide level to high tide level. Diversity and distribution of crabs were significantly different between the sites and among tidal levels. A total of 13 species of brachyuran crabs were recorded belongs from the family Ocypodidae, Grapsidae and Varunidae in the Bhaira mangroves, while 10 species were recorded in Miani mangroves. The abundance of crabs also varied between the two mangrove habitats (32–64 per meter square in Bhaira mangroves and 26–212 per meter square in Sonmiani mangroves).

STUDY OF BIO-ECOLOGICAL STATUS, MANAGEMENT AND CONSERVATION OF MARSH CROCODILES (*Crocodylus palustris*) IN SINDH

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The Crocodylians (Crocodyles, Alligators, and Gavials/Gharials) are well-known and wide spread occupants of tropical and subtropical habitats. There are 23 species of Crocodyles in the world today. Marsh Crocodile (*Crocodylus palustris*) is the only species that has been recorded in Sindh Pakistan, occurring in Mangho Pir, Karachi Zoological Garden and Khar Center (Karachi), Haleji Lake (Thatta), Chotiari Reservoir (Sanghar), Deh Akro 2 (Nawabshah), Nara Game Reserve (Khairpur mirus) and New Jatoti (Noshehroferroze). Present study on the Bio-ecological status and distribution of Marsh Crocodile or Mugger (*Crocodylus palustris*) was carried out from January 2006 to June 2009. According to the Red List of IUCN the Marsh Crocodile is considered to be threatened and endangered species in Sindh Pakistan. The ecological factors were analyzed to identify the impacts of decline in Marsh Crocodile population due to indiscriminate habitat destruction, environmental problems, low water quality and hunting pressure. The physico-chemical parameters study was carried out several sites to know the ecological status and water quality of species.

**STUDY ON MALOCCLUSION OF TEETH OF VARIOUS SPECIMENS OF
RODENTS OF DIFFERENT BIOTOPES**

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All the collected no of 500 specimens of rodents including male and females used in a study of abnormality of teeth. The reported no of 80 rats were examine with enlarge incisor of upper and lower jaw Other more than 50 rodents continued grow with their fully carves like a ring shape structure. Such rodents having the malocclusion of teeth have to seen the weak and lose the body weight comparison with other members.

**EFFECT OF VARYING PROPORTIONS OF MAIZE GLUTEN AND RICE
BRAN AS FEED SUPPLEMENT ON THE GROWTH OF CARPS AFTER
YEARLINGS STAGE IN COMPOSITE FISH CUL TURE SYSTEM**

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To evaluate the potential of maize gluten and rice bran as food supplement in pond fisheries; three fish species viz. *Labeo rohita*, *Cirrhinus mrigala* and *Ctenopharyngodon idella* were stocked in the ratio of 100:50:25 at the rate of 1120 fish/ha for a period of six months in two earthen ponds P₁ and P₂, respectively each having dimensions of (16 x 30 x 1.5m) located at University of Agriculture Faisalabad. These fish were provided with maize gluten and rice bran with the ratio of 3:2 and 4: 1 in P₁ and P₂ respectively at the rate of 1 % of wet fish body weight daily. Overall net fish production of these two experimental ponds remained statistically significantly different to each, other with mean values as 3336.04 and 1975.11 Kg/ha/year in P₁ and P₂, respectively. P₁ which was supplemented with maize gk'ten and rice bran with the ratio of 3:2 showed significantly better growth of fish as compared to P₂ which was supplemented with the same ingredients at the ratio of 4:1. Throughout the experimental period *C. idella* performed better in terms of weight gains as compared to *L. rohita* and *C. mrigala*. Various water quality parameters such as dissolved oxygen, water temperature, light penetration and electrical conductivity were also monitored fortnightly for the whole study period. Interpond variations were analyzed through analysis of variance. Intrapond correlations among various ecological factors were also estimated.

ADDITIONAL REPORT ON *ENCEPHALOIDES ARMSTRONGI* WOOD-MASON, 1891 (DECAPODA, MAJIDAE, INACHINAE) FROM PAKISTANI WATERS

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Encephaloides armstrongi Wood-Mason is the only species in the genus. It is the most distinctive majid crab in the Indo West Pacific at 170-311 m depth. Till now it was not collected from the Pakistani waters, thus the present record fills the gap in the distribution of species. The present specimens were collected from the fishing area of Karachi at 120m depth. The species densely found in the oxygen minimum zone of the Arabian Sea. (Bett, 1995). It is fully illustrated and described in the present - paper the vertical distribution and density relationship is also discussed.

STOMACH CONTENTS OF THE COMMERCIAL CRABS *PORTUNUS PELAGICUS* AND *P. SANGUINOLENTUS* (CRUSTACEA, DECAPODA, PORTUNIDAE) FROM THE COASTAL WATERS OF KARACHI

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A total of 558 *Portunus pelagicus* (Linnaeus, 1766) and 426 *P. sanguinolentus* (Herbst, 1783) were examined for stomach contents. These crabs were collected from the commercial landings at Korangi Fish Harbour, Karachi from January 2004 to December 2005. Out of 558 *P. pelagicus* only 254 (or 45.52 %) crabs had empty stomach while 304 (or 54.48%) crabs had food remains in their cardiac stomach. Bivalves (27.6 percent point), crustaceans (27.01 percent point) and gastropods (12.6 percent points) were the main food items found in the stomachs of *P. pelagicus*. In terms of frequency of occurrence the order of relative importance of bivalves, crustaceans and gastropods was the same as that of points method. Bivalves occurred in 34.5% stomachs while crustaceans and gastropods occurred in 29.86 % and 24.01 % stomachs. In case of *P. sanguinolentus*, the stomachs of 227 (or 53.29%) crabs were found empty while 199 (or 46.71%) crabs had food remains in their stomachs. Crustaceans were found as the most commonly ingested food items in the diet of *P. sanguinolentus* with a score of 28.0 percent point. Bivalves ranked second highest food category with a score of 16.4 percent points. According to percent occurrence method the most frequently occurred food item was found to be crustaceans (31.2%) followed by bivalves (24.6%) and gastropods (15.6%). Fish, echinoderms and algae were ingested in small amounts and were found in few stomachs. The two species of *Portunus* appear to have similar feeding habit and occupy the same habitat. Hence the inter-specific competition for food and space may occur between *P. pelagicus* and *P. sanguinolentus*.

DIVERSIFIED FEATURES OF A NASSARIID GASTROPOD, THE SANDY-BEACH WHELK "*BULLIA*"

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Although gastropods are usually habitant of rocky shores but there are certain species which inhabit the sandy and muddy substratum. Among the gastropods, living in sandy and muddy substratum are the members of the Family Nassariidae which are commonly called as sandy beach whelk. The genus *Bullia* is a member of Family Nassariidae. *Bullia* may be divided into two groups ie., those which are predominantly intertidal and exploit wave action and CUITents by spreading their broad, thin, agile feet and surfing up and down the shore, and those which are chiefly or entirely subtidal and do not surf. The species of *Bullia* are blind and even a single species acquire a wide range of shell color variation from white to dark violet. They are typically carnivorous scavengers, feeding mainly on dead or dying animal matter and can survive for a month in laboratory without feedilig. The sexes are separate and fertilization is internal. In both males and females the gonads are intermingled with digestive gland.

OCCURRENCE OF SNAILS IN SOME WHEAT FIELDS OF FAISALABAD

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Characterization of mollusks habitants regarding, number, diversity and living strategies is very informative for biodiversity studies. High input crop fields (cultivation with intensive farming using pesticides and synthetic fertilizers) and low input crop fields of wheat were selected at random from an area of 10 acre, for the collection of mollusks. Soil samples were taken from three different microhabitats within the wheat fields from the early stage of growth of wheat crop till the harvesting for total duration of 5 months. The snail diversity and abundance in wheat fields varied with months. There was least diversity of snails in the month of May while it was maximum in the month of January followed by February. *Monadenia fide/is* was found to be the most frequent species throughout a period of five months and under all the microhabitats, followed by *Oxychillus cellar ius* and *Oxychillus draparnaudii*. Out of 271 specimens, 89 specimens were found on the edges of the wheat field while 179 specimens were found under trees; only 3 specimens were found in the mid field area. The diversity of snails on the edges and under the trees was almost similar and there was not a significant difference. Seventy five specimens under were found the Baans tree, 61 Dhreek tree, 30 specimens were found under Sheesham tree, whereas under Arind, Shehtoot, Guava, and Mango tree there

were found 4,3,1,1 specimens, respectively. From this we can conclude that possibly there is independent chemo sphere of all the plants in their surroundings which may also effect snail diversity. A total of 271 snail specimens representing 8 genera and 7 families of Phylum Mollusca occurred in low input wheat fields.

**FIRST REPORT OF THE FOSSIL ECHINOID *HISTOCIDARIS* SP.
MORTENSEN, 1903 (ECHINOIDEA: CIDAROIDEA: HISTICIDARIDAE) FROM
PAKISTAN**

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Few specimens of regular echinoid species *Histocidaris* sp. Mortensen, 1903 have been found in the collection of fossils obtained during the survey of Thana Bula Khan (Pakistan). There are around 800 extant echinoid species and the group has a long and detailed fossil record stretching back about 450 million years ago to the late Ordovician period. The genus *Histocidaris* is now reported from Pakistan for the first time. It is being briefly described here with a range from the Miocene to the recent.

***CHANNA MARULIUS* AS CONTROLLING PREDATOR IN THE CULTURE OF
TILAPIA AND ITS GROWTH PERFORMANCE**

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The *Channa marulius* was stocked with *Tilapia* at different predatory prey ratio and it had to be checked that how many *Channa marulius* are required to control prolific breeding of *Tilapia*. It was found that increase in the predatory prey ratio enhanced the fish production. The present experiment was conducted to study *Channa marulius* as controlling predator in the culture of *tilapia* and its growth performance in different ratios in the fertilized ponds for the period of five months. Four ponds were selected at Fisheries Research Farms, University of Agriculture, Faisalabad. Fertilization of both ponds was done with cow manure. In all the four ponds *Tilapia* was also added as food for *Channa marulius*. After preliminary preparations these ponds were stocked with the stocking density as: pond 1 *Tilapia* were 20 in number, pond two 40, pond 3 there were 60 *Tilapia* and in pond four there were 80 *Tilapia* whereas the number of *Channa marulius* was kept constant i.e. 10 in all the ponds. At the time of stocking average body weights of *Channa marulius* for pond 1 was 275.4g, for pond 2 it was 270.6g, for pond 3 it was 186.7g and for the fourth pond it was 208.3gm. Average total lengths, at the time of

stocking, for pond 1 was 21.44cm for the pond2 it was 24.1 cm, for pond 3 it was 20.5cm and 23.2cm for pond 4. Water samples of all ponds were analyzed at monthly intervals for carbonates, bicarbonates, total hardness, biomass, calcium and magnesium. At least 5 fishes were captured from each pond at monthly intervals and two parameters i.e. body weight and total length was recorded. It was concluded that average increase in body weight of *C. marulius* was 40.3g in the pond 1, 40.5g in the pond2, 46.9g in the pond 3 and in the pond 4 it was 50.5g. Similarly, average increase in total length was 4.8cm in the pond 1, 4.1cm in the pond 2, and 4.6cm in the pond 3 and 4.2cm in the pond 4. Under the said poly culture system *Tilapia* was found to be suitable forage fish for *Channa marulius* and can be cultured in even more ratios.

FISHES OF THE RIVER ATTOCK GORGE WITH NEW RECORD OF *BATASIO PAKISTANICUS*

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River Indus takes its origin in the south western Tibet. It enters in district Attock at Momanpura. It contracts in to a narrow rocky bed after joining of Kabul River on its right bank. It rushes on through a gorge with a high bank on each side, having Attock Fort on its left bank. Below Attock Khurd near Bagh Nilab it again spreads out into a kind of lake. But soon again contracts and flows through a gorge down to Makhad and hence out beyond the district boundaries after flowing about 95 Km. Total 31 number of fish species belonging to 28 genera 12 families and 6 orders have been collected with a new record of *Batasio pakistanicus* (Family Bagridae). Previously, this species was described from Jinnah Barrage in the Mianwali District. Its new locality is situated about 150 km upstream.

PRELIMINARY STUDY TO ASSESS THE SURVIVAL RATE OF JUVENILES BANANA PRAWN (*PENAEUS MERGUIENSIS*) AT DIFFERENT SALINITIES

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The study was conducted to examine the salinity tolerance in the juveniles of penaeid shrimp, *Penaeus merguensis*. This species is suitable for aquaculture and is abundant in Pakistani waters. For this purpose juveniles of *P. merguensis* were collected from Miani Hor Lagoon which is an important fishing centre along the Balochistan coast. On arrival to lab, the juveniles were first acclimatized for 36 hours (39ppt, 30°C). The

size of juveniles ranged from 5.53cm to 4.73cm and weight 1.28g to 0.54g. After acclimatization 12 juveniles were exposed to salinities ranging from 5‰ to 50‰ in 5‰ increments. All experiment was conducted at temperature $30 \pm 1^\circ\text{C}$. Survival rate was checked after 1, 3, 6, 24, 48, 72, 96 hours. No feed was provided during the experiment. Survival rate was 58%, 94.4% and 75% at 5‰, 10‰ and 15‰ respectively. While no mortality was observed at 20‰ to 50‰. The present study showed that juveniles can tolerate wide range of salinity.

MERISTIC AND MORPHOMETRIC STUDIES OF INDUS MAHSEER *TOR MACROLEPIS* (HECKEL) (TELEOSTEI: CYPRINIDAE) FROM ATTOCK, PAKISTAN

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Among Mahseers, Indus Mahseer *Tor macrolepis* is the important game and food fish of Pakistan. For the study, one year fish sampling was conducted at various sites of Attock district and adjoining areas from 2008 to 2009. For the purpose, Haro River was divided into four sampling zones, each consisting of about -10 km area. Fifth sampling zone was selected in the Hasan Abdaal area. During this period, 118 specimens were caught from these five sampling zones. More than forty important morphometric and meristic parameters were selected for the study.

STUDY OF FODDER WEED COMPLEX AS HARBORAGE OF PREDATOR ARTHROPODY

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NADEEML

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With in the agroecosystem the provision and manipulation of shelter habitat features provide an approach for enhancing the effectiveness of natural enemies for biological pest control. The study was carried out in the suburbs of Faisalabad city, ranging from 30 to 60 km distant from University of Agriculture, Faisalabad. During the study period (Nov.2008 to May, 2009) a total of 134 Arthropods specimens were collected from three Fodders crop weeds i.e. *Avena fatua*, *Cnicus* and *Ageratum conyzoides*. Out of this 83 specimens were declared as predators. *Solenopsis invicta* is reported as key stone species during the sampling season and it is very important in

controlling the number of other insect arthropods. Environmental factors like temperature, humidity and duration of day light have great effect on diversity and abundance of arthropods predator fauna. Total 1266 specimens were captured out of which 602 specimens from edge and 664 from centre. Order Coleoptera remained dominant order from December to May, 2009 during the whole sampling season. As a whole it was observed that predator arthropods are more abundantly found in the centre of the fodder crops under study as compared to the field margins: Shannon-Weiner Index and t-test diversity for arthropods was checked. This study gave preliminary information on the fate of faunal diversity. There is a dire need of future study in this respect because such studies have been reported to have significant and direct benefits to the farming industries.

STUDIES ON RELATIONSHIP BETWEEN SEASON AND INORGANIC METALS IN THREE LAKES OF AZAD JAMMU KASHMIR

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The present study was carried out at three lakes of Azad Jammu Kashmir, from June 2007- Feb 2008. Study area was divided into three sites, site I (Chikar lake), Site II (Banjosa lake) and Site III (Bagh lake). Three samples from the surface of water were taken from these three sites on seasonal basis (summer, winter). Main objective of this study was to evaluate water quality of these sites in relation to metal ion concentrations. Water samples were analyzed for inorganic metals (Cd, Cr, Ni, Cu, Pb, Ca and Mg) by using atomic absorption spectrophotometer. In site I Cd showed deviation from standard values of Pakistan Standard Institution (PSI) while other parameters are within limits. Metal ion concentrations in Site II, III were within favorable limits. ANOVA showed non significant difference between three sites.

CLIMATE CHANGE AND VULNERABLE COASTAL ECOSYSTEMS OF PAKISTAN

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Coastal Ecosystems of Pakistan are one of the richest and biodiverse ecosystems. The coastal areas of Sindh and Balochistan are regions of high economic value where socioeconomic activities are highly concentrated. Much of the coastal elevation of Sindh

is close to the astronomical high tide and is vulnerable to rising sea level. The Km 320 long coastal area of Sindh is a passive margin and a home for the fifth largest mangrove population, whereas, the Km 670 coastline of Balochistan is a subduction zone having high ocean productivity. The impacts of climate change, such as rising sea level, incremental increase in air and sea surface temperatures, and increased frequency of extreme climatic events can directly and indirectly impact the productive potential of the coastal ecosystem. Climate variability threatens to disrupt the environment of the ecoregion not just one species at a time, but by disrupting the physical features and ecosystem functions that support biota. Once the threats and impacts have been identified, monitoring the threats would then be essential in determining how the ongoing process of climate change will affect the coastal ecosystem. The paper highlights some of the threatened and vulnerable coastal marine ecosystems of Pakistan to climate variability.

DISTRIBUTION OF SMALL MAMMALS OF BANNIGALA HILLS ISLAMABAD

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Study was conducted to determine the distribution of small mammals in Bannigala Hills during June to November 2006. Bannigala Hills comprises an area of about 205 hectares situated on the right side of Rawalpindi - Murree road, about 4 kilometers from Bhakkar. During the study, 10 species of small mammals belonging to four orders, nine families and 10 genera were identified. Among these, two species belong to Order Insectivora (*Paraechinus hypomelas* and *Suncus murinus*), two species (*Rhinolophus ferrumequinum* and *Hipposideros fulvus*) from Chiroptera, one species (*Lepus nigricollis dayanus*) from Order Lagomorpha and five species (*Funambulus pennantii*, *Hystrix indica*, *Mus musculus*, *Gerbillus nanus*, *Tatera indica*) from Order Rodentia. Trapping method was used to collect some of the mammals from different habitats such as sandy area, rocky area, slopy hills, plain area and water channels. However, the presence of some other mammals was confirmed through indirect evidences (droppings, quills and borrows) in study area. *Mus musculus*, *Lepus nigricollis* and *Hystrix indica* were found common in study area. *Suncus murinus*, *Funambulus pennantii* and *Gerbillus nanus* were not very common in the area. Similarly, *Paraechinus hypomelas*, *Rhinolophus ferrumequinum*, *Hipposideros fulvus* and *Tatera indica* are considered susceptible species in the area. Habitat destruction due to deforestation, overgrazing, grass cutting, fuel wood extraction and logging inside the study area are the major threats to the existing wildlife.

**LIVESTOCK DEPREDATION BY COMMON LEOPARD-AN ALARMING
INTIMIDATION FOR ITS CONSERVATION IN MACHIARA NATIONAL
PARK, PAKISTAN**

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Carnivorous animals are not acceptable for humans due to depredation of their livestock. Conflict becomes severe when carnivores species is critically endangered like that of common leopard. Machirara National Park (MNP) is protected area, where livestock depredation increasing that pose a threat for the management and conservational efforts of leopard. The study was aimed to assessment of livestock depredation by leopard and its negative effects on its conservation. Results showed that during 200-2008 a total of 183 animals were killed including goats (45%), sheep (39%), poultry (5%), cattle (2%) and equine (1%). Maximum killing were occurred in May (32%) followed by June (9%) and March (8%). Majority of livestock were killed in 8am-12pm (46%) followed by 4pm-7pm (20%) and 7pm-10 pm (11%). Most of livestock (48%) were killed at 0-100m distance from forest, followed by 500-1000m (21%) and 100-500m (20%). Valleys were maximum killing sites (48%) followed by steep slopes (28%) and gradual slopes (20%). Only 6% livestock were killed inside houses. 58% people made loud noise against leopard attacks, 11% try to chase it while 5% used marriage bombs to keep it away. Maximum injuries (25%) occurred in July, followed by Nov (16%) and August (15%). 44% attacks were accidental. People aptitude toward leopard showed that 90% people want to illuminate this species from the area, whereas 10% people thought that it is useful animal because of its pellets. Results showed that additional steps would have been taken to conserve this endangered species. Education and awareness programs should be started to make people aware from leopard's importance and best herding practices to avoid their livestock lose.

**INFLUENCE OF MAJOR CARPS ON GROWTH POTENTIAL OF EXOTIC
CARPS IN SEMI-INTENSIVE CULTURE SYSTEM**

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The experiment was carried out to evaluate the effect of Rohu (*Labeo rohita*) on growth potential of exotic species viz. *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* in semi-intensive polyculture system at Fisheries Research

Farms, University of Agriculture, Faisalabad, in two earthen ponds for duration of 160 days. Four fish species viz. *Labeo rohita*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* were stocked in pond one with equal ratio of 20 of each species while in pond two, three fish species viz. *Cirrhinus mrigala*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* were stocked with equal ratio of 30 of each species. Both the ponds were fertilized with poultry manure at the rate of 0.08 % of wet fish body weight daily and NH_4NO_3 and single super phosphate at the rate of 0.008g N/g of the wet fish body weight daily. The result showed that overall total production was not influenced by the addition of major carps in experimental ponds. Nitrogen incorporation efficiencies (NIE) of both ponds were 6.14 and 4.00 and Nitrogen conversion efficiencies (NCE) were 0.11 and 0.9 in pond one and two respectively. The net production of fish remained as 3131.50 and 2600.900Kg/ha/year in experimental pond one and two respectively.

PLANT DIVERSITY IN THE CHOLISTAN GAME RESERVE, PUNJAB, PAKISTAN

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Cholistan desert is of immense importance with regard to some very important game mammals and birds. The wilderness of Cholistan has its own charms and beauty and has the fame of a rewarding hunting ground. Chinkara (*Gazella bennetti*), blackbuck (*Antelope cervicapra*), nilgai (*Boselaphus tragocamelus*), houbara bustard (*Chlamydotis undulata*), great Indian bustard (*Ardeotis nigriceps*), grey partridge (*Francolinus pondicerianus*), imperial sandgrouse (*Pterocles orientalis*) and common quail (*Coturnix coturnix*) are some of the important game species and attract the attention of local hunters as well as foreign dignitaries, specially from the Middle East, who visit Cholistan to practice falconry on houbara. To investigate the biodiversity of plant species Cholistan was explored during October 2000. Soil of the desert was predominantly sandy to sandy loam. Vegetation was studied at 26 different sites in Lesser Cholistan and 20 sites at Greater Cholistan along jeepable tracts and data were recorded for relative frequency, relative density, relative cover and importance value of each plant species. A total of 2 tree species, 8 shrub species, 3 under-shrub species, 23 herb species, 19 grass species and solitary sedge species were recorded during survey. Dominant species were *Aristida adscensionis*, *Cymbopogon jwarancusa*, *Ochthochloa compressa*, *Lasiurus scindicus*, *Sporobolus iocladius*, *Cenchrus biflorus*, *Aeluropus lagopoides*, *Callignum lagopoides*, *Crotalaria burhia*, *Suaeda fruticosa*, *salsola baryosma*, *Leptadenia pyrotechnica*, *Haloxylon recurvum*, *Haloxylon salicornicum*, *Dipterygium glaucum*, *Zaleya pentandra* and *Trianthema triquetra*.

PHYTO-SOCIOLOGICAL STUDIES OF LEHRI-JINDI GAME RESERVE, JHELMUM

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Lehri / Jindi Reserved Forest is about 6 km from Dina on a way to Rawalpindi between 73°27' and 73°38' longitude and 33°00' and 33°13' latitude. Total area of these two forests is about 80 sq km. The vegetation is typically subtropical dry evergreen open scrub, comprising mainly of few shrubs and many annual and perennial grasses. The habitat is suitable for many important wildlife species, including potential game species like Punjab urial (*Ovis vigne punjabiensis*), guldar (*Panthera pardus*), wolf (*Canis lupus*), hare (*Lepus nigricollis*), black francilin (*Francolinus francolinus*), grey francolin (*Francolinus pondicerianus*), seese (*Amoperdix griseogularis*) and chakor (*Alectoris graeca*). Vegetation is a typical subtropical open scrub type but highly depleted and some immediate steps should be taken to improve the habitat and ultimately the wildlife of these forests. Controlled grazing and woodcutting is allowed inside the forest area is advised. Planting of native species will certainly help in improving the present state of vegetation cover. New introductions of useful species should be made after careful studies, however, *Olea ferruginea*, *Dalbergia sissoo*, *Punica granatum* and *Pyrus pashia* may prove valuable. Provided the present level of human interference, they will no more be able to sustain in the long term future. Vegetation assessment survey was conducted in October 2001 to explore the present state of biodiversity at Lehri / Jindi Reserved Forest area. Vegetation cover is about 60 %. Trees and large shrubs contributing only 20-30 % cover. However, plant diversity is astonishingly high; over 140 species were recorded during a single season survey belonging to 46 families.

ASSESSMENT OF HOARDING OF WHEAT GRAINS BY *BANDICOTA BENGALENSIS* IN CHEHLA BANDI, MUZAFFARABAD, AZAD KASHMIR, PAKISTAN

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A study was conducted to determine and assessment of hoarding of wheat grains by *Bandicota bengalensis* in burrows near Chehla Bandi during May 2008. Chehla Bandi is situated at about 2.5km North to Muzaffarabad city, the capital of Azad Jammu and Kashmir (Pakistan). *B. bengalensis* is the most serious rodent pest of crops in AJ&K.

Burrows of *B. bengalensis* are characterized by the presence of large heaps of loose soil and open mouths of burrow system. Wheat cut tillers were found all around the burrow mouth. The length of the four burrows varied from 1.82 to 5.48 (average 4.1 ± 0.84) and the width of the four burrows varied from 1.21 to 1.92 (average 1.6 ± 0.15). Insects and weeds were also present inside the burrows. Burrows density was also calculated during the study, total 104 active burrows were present in 1.65 hectares in the study area. Ten samples were taken to compare the loss done by *B. bengalensis* in the study area. The mean weight of grains was 225.30 ± 2.87 g and the mean weight of hoarded material was 477.20 ± 57.9 g and number of spikes hoarded by the *B. bengalensis* was 660 ± 179 . Economic loss done by *B. bengalensis* showed highly significant difference (cal.t 8.707 at 9d.f. and 0.05p) between wt. (g) of control panicles. The present study showed that the *B. bengalensis* hoarded a large number of wheat in their burrows and destroyed the wheat fields at a very large scale. In the post harvest period, it is important to eliminate crop trash, for example, by burning, and to plough the fields as soon as possible in order to disrupt any residual food supply that is left in the fields.

PROSPECTS AND LIMITATIONS OF CATTLE PRODUCTION IN PUNJAB, PAKISTAN

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A total of two hundred (300) respondents were interviewed from the selected districts of various zones of Punjab during a survey, to study the prospects and limitations of cattle production kept under different production systems. Cattle production in Punjab is being practiced under four different systems viz; rural barani(RB), rural irrigated (RI), peri-urban (PU) and Progressive (Prog) farming respectively. Only those respondents were interviewed from various districts located in different parts of Punjab province where cattle production was the main source of income. The field data collected through personal face-to-face interviews by field teams were analyzed using computer software "SPSS". The results indicated that 26.5% farmers were illiterate, 51.9% were matriculate and only 21.6% were inter or above qualified. Within this education status, the proportion of agriculturists was 77.2% while 22.8% were land less non-agriculturists but were depending on cattle production to earn their livelihood. It was observed that on overall basis 5.82 lactating, 6.13 dry, 4.41 breeding males and 2.20 draught purebred cattle were kept by the respondents. The number of crossbred cattle kept by the respondents on overall basis were 5.61, 4.53, 1.50 and 3.69, respectively for above mentioned categories. The non-descript lactating cows, dry cows, breeding males and draught cattle were 6.83,

12.33, 5.08 and 2.63, respectively. About 47.9 % farmers demanded for the provision of better infrastructure and improvement in marketing system, while 17.1 % suggested the need of specific livestock market. Whereas 30.8 % pointed out the need for provision of credit facilities and 4./3 % demanded the establishment of market with proper legislation. Various practices regarding feeds and fodder adopted by the cattle farmers have been presented. It can be concluded that a lot of improvement is possible in buffalo production through the adoption of proper feeding and management practices under field conditions. Majority of the farmers were not aware of many technological interventions, which indicated the need of establishing an efficient livestock extension services.

A STUDY ON BREEDING PRACTICES OF SMALL RUMINANT KEPT UNDER VARIOUS PRODUCTION SYSTEMS IN PUNJAB, PAKISTAN

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A total of two hundred and fifty (250) respondents were interviewed during a survey conducted in Punjab, Pakistan to study the breeding practices of small ruminant kept under different production systems. The current small ruminant production systems in Punjab are Transhumant (TH), Sedentary (SD), Household (HH) and Commercial (Com) farming respectively. Only those respondents were interviewed from various districts located in different parts of Punjab province where small ruminant production was the predominant source of livelihood. The field data collected through personal face-to-face interviews by field teams were analyzed using computer software "SPSS". Intensity of different methods used for breeding small ruminants in the various production systems were : by breeding ram/buck owned by the farmers themselves (72.7%), neighbor's (41.9%), relatives (41.9%) and service provider (15.1%) respectively. When farmers were asked the reasons for maintaining their own breeding males, on overall basis, 29.4% claimed that their rams were superior, 58.2% said that their own males were available at all the needy times and 12.4% farmers mentioned that maintaining own rams/bucks was less expensive. The average values for age at maturity (AM) were 9.89 and 13.89, 11.14 and 13.01, 10.49 and 12.11, 9.00 and 13.50 months in TH, SD, HH and Com production systems of sheep and goat, respectively. While on overall basis the corresponding figures in sheep and goats, were 9.89 and 13.89 months. The average values for lambing interval (LI) and kidding interval (KI) were 11.0 and 12.11, 9.99 and 9.44, 10.10 and 9.54 and 7.50 and 8.50 months in TH, SD, HH and Com production systems of sheep and goat, respectively. While on overall basis corresponding

figures in sheep and goat, were 11.0 and 9.61 months. The services of A.I. in small ruminants are overwhelmed in Pakistan due to non-availability of infrastructure (no semen unit) which can provide superior semen to motivate the farmers to adopt A.I.

**ATTITUDE OF CAMEL HERDERS TOWARDS INTERVENTIONS
REGARDING CAMEL CALF HEALTH CARE AND MANAGEMENT
PRACTICES UNDER PASTORALISTS CONDITIONS**

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A survey was conducted in District Jhang of Pakistan. Pastoralists were divided into small, medium and large camel herders on the basis of camel population. Forty eight camel herds owned by 16 pastoralists from each category were selected randomly to study the status of health care and management of camel calves. The results showed that the mortality rate in camel calves was 65.8%. None of the camel herders was cutting and disinfecting the navel cord and a large number of them (87.8%) fed colostrums after the expulsion of placenta. Intensity of occurrence of different diseases in camel calves revealed maximum intensity of mange (85.4%) followed by diarrhea and pneumonia (70.8%), camel pox (37.5%) and anthrax (16.7%). A majority (60.4%) of these herders did not consult a veterinarian for the treatment of sick calves and 83.3% camel herders were feeding colostrum to camel calves after the expulsion of placenta. This study tended to show that these herders were not interested in rearing the male calves because they did not expect sizeable returns from their sale. Thus, there is an urgent need to educate these camel herders to make camel calf rearing an economical proposition.

**STUDY ON BREEDING PRACTICES OF INDIGENOUS CATTLE KEPT
UNDER VARIOUS PRODUCTION SYSTEMS IN PUNJAB, PAKISTAN**

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A survey was conducted in Punjab, Pakistan to study the existing cattle production systems in Punjab. A total of 200 respondents raising cattle were interviewed from

various districts located in different parts of Punjab province. The cattle production is being practiced under four production systems viz. rural barani (RB), rural irrigated (RI), peri-urban (PU) and Progressive (Prog) farming. The field data collected through personal face to face interviews by field teams was analyzed using computer software "SPSS". It was concluded there were only 37.8% of the cattle producers who had used A.I. The other 13.9% used both natural and A.I. service, whereas 48.3% used natural services for breeding of their animals (mostly own superior bulls (4.6%). The average age of non-descript cattle at maturity was 35.5 months, of poor bred cattle 32.4 months and of crossbred 20.1 months. It indicated that a high percentage of farmers preferred natural services by the breeding bull to A.I. due to non availability (13.8% cases) of A.I, low conception with A.I. (75.4% cases) and more cost (6.2%) of A.I facility. It may be suggested that adequate infrastructure of A.I facility including technical skilled man power, proper handling of the semen, cost effective quality semen and least but the last, self training of the farmers in A.I rather than unskilled A.I technician may be one solution to get fruitful result from A.I facility

MILK PRODUCTION POTENTIAL OF PAKISTANI CAMEL (*CAMELUS DROMEDARIUS*) UNDER THE DRY LAND CONDITIONS OF THE PUNJAB

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The study was undertaken to assess the dairy character of single humped non-descript (locally called as Desi) camels raised under dry land conditions. Animals belonged to the Barani Livestock Production Research Institute (BLPRI) Kherimurat, District Attock (Punjab-Pakistan). Milk production of five she-camels was recorded monthly from 7th day post-calving till the completion of six months lactation. The browsing/grazing for these animals in the mountainous rangeland mainly comprised of Pholai (*Acacia modesta*), Kandair (*Alhaji camelorum*) and Kao (*Olea ferruginea*) trees and some local grasses like Palwan (*Olea cuspidata*), Chita (*Cymbopogon distance*) and Sariala (*Heteropogon contortus*). Common salt and water were provided *ad-libitum*. All the camels were hand milked after the calves suckled the udder. Experimental animals produced 2100.61 ± 163.32 litres milk in a period of 180 days. The mean milk production was 11.66 ± 0.90 L/d, with the peak milk production in the second month of lactation. The results of the study revealed that camel possess an appreciable dairy potential even under the ranges or dry land conditions. This can be further exploited under good feeding and management conditions coupled with their careful selection and breeding. Conclusively being a member of food producing family, through the application of biotechnology for instance embryo transfer, camels' milk production can significantly be improved. This step will certainly bring prosperity for the people towards their sustainability, particularly belonging to arid/semi arid areas. Moreover undertaking

certain biomedical studies on camel milk can prove this animal more vulnerable among the various farm animal species.

MASCULINEZATION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) THROUGH ANDROGEN SEX REVERSAL

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Sex reversal by using androgen; 17 alpha methyl testosterone (MT) is a widely used technique in order to produce all male progeny in tilapia. In the present study effect of MT for different time duration i.e. 15, 20, 25 and 30 days on sex reversal (masculinization) and growth performance of Nile tilapia was evaluated. MT was administered orally by using 40% crude protein diet to tilapia fry @ 70 mg MT/ kg of feed in glass aquaria for the selected time durations. After this period fry was shifted to earthen ponds to monitor its growth performance for 280 days (20 fortnights). At the end of experiment the sex ratio was determined by examining gonads after dissecting the fish. Growth performance was monitored by recording the morpho-metric characteristics i.e. wet body weight and total length of fish on fortnightly basis. All MT receiving treatments showed a statistically significantly higher male proportion than control. Treatment duration of 25 days resulted in maximum male population (98%) with no female and 2 % intersexes. Treatment duration of 15, 20 and 30 days resulted in 91.7, 92 and 93 % males respectively. The treatment duration of 25 days gave the maximum fish production i.e. 3749 kg/ ha./ year, 1.8 times greater than control.

STUDY OF POPULATION STATUS AND ECOLOGICAL REQUIREMENT OF THE MARSH CROCODILE (*Crocodylus palustris*) IN CHOTIARI RESERVOIR, SANGHAR DISTRICT, SINDH

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The Marsh crocodile (*Crocodylus palustris*) has adopted itself to the terrestrial life very skillfully. In fact, it is believed to be more mobile on land and can move over substantial distances while finding a more suitable habitat. Marsh crocodile of Sindh is known to dig burrows as shelters during the dry season. The study was carried out in the Chotiari Reservoir which is situated at 20 km North East from Sanghar city, at 69°.4 E longitudes and 26°.1" N latitudes. It covers an area of 86 km². The reservoir has been

constructed on the east side of the Nara Canal. The chotiari reservoir is filled by the Ranto canal in the flood season. The study on the status and distribution of the Marsh crocodiles (*Crocodylus palustris*) was carried out from November 2007 to September 2008 during which crocodiles were recorded from Chotiari Reservoir and its surroundings with an estimated population of Marsh Crocodiles. Previously, the prime source of threat to (*Crocodylus palustris*) was the construction of irrigation networks and illegal skin trading, of which the latter has been halted by international treaties such as the Convention of International Trade of Endangered Species (CITES). Now current threats have changed to habitat destruction, through anthropogenic activities and some extent reduced water release up-stream of their habitat. Marsh crocodiles can be ranched, but the concept of sustainable utilization of wildlife in general is still anathema to a large segment of the population in Chotiari reservoir. On the other hand, if crocodiles could benefit people, they are not likely to become extinct. This paper looks at the population of Marsh Crocodile at Chotiari Reservoir and surrounding areas and suggests certain conservation measures for their in-situ and ex-situ protection.

POPULATION STATUS AND DISTRIBUTION PATTERN OF RED JUNGLE FOWL (*GALLUS GALLUS*) IN DEVA VATALA NATIONAL PARK, AZAD JAMMU & KASHMIR

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A study was conducted to determine the population and distribution pattern of Red Jungle Fowl (*Gallus gallus*) in Deva Vatala National Park, Azad Jammu and Kashmir, during April- September, 2008. For data collection, study area was divided in to four localities/sites i.e. Chumb, Deva, Burmala and Vatala. Line transects and call count methods were used to estimate the population of Red Jungle Fowl at study sites. A total of six line transects were laid in each locality both during the morning and evening hours. A population of 48 individuals of Red jungle fowl was estimated in the study area having an overall density of 5.43 adult birds/ km². However, the bird density varied at individual sites having 7.23, 3.67, 6.67 and 5.34 adult birds/ km² in Chumb, Deva, Burmala and Vatala localities, respectively. In the study area, red jungle fowl occupied an altitudinal range between 351m and 411m, preferring sloppy and densely vegetated areas. The birds preferred the areas with grassland habitat followed by the habitat having plant species such as *Zizyphus jujuba* and *Lantana camara* as dominant vegetation. Red jungle fowl seems to have pushed back into most densely vegetated areas of the park mainly due to human disturbance being caused by local hunters, shepherds and grass cutters. Results of this preliminary study would be useful in conservation planning of this threatened species in Deva Vatala National Park.

**LENGTH WEIGHT AND CONDITION FACTOR RELATIONSHIP OF WILD
MYSTUS BLEEKERI FROM SIALKOT, PAKISTAN**

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One hundreds and five samples of wild *Mystus bleekeri* of different body size ranging 5.5 - 7.8 cm total length and 1.20 – 3.02 gm body weight were used for the analysis of morphometric variable of head length, head width, dorsal fin length, body girth, body depth, tail length and width in relation to total length and body weight of the fish to investigate allometric growth. It was observed that all these relations showed very high correlation's. Slopes of the log transformed data were used to compare with an isometric slope ($b = 1$ or $b = 3$), It was found that all the parameters examined showed isometric growth in relation to either total length or body weight, growth in weight is almost proportional to the cube of its length, the values of the slope ($b = 0.30$) which shows isometric growth. Regression parameters were found to be highly significant.

**SEASONAL DYNAMICS, SLOPE ASPECT AND LAND USE EFFECTS ON SOIL
FAUNAL POPULATION DENSITY AND DIVERSITY IN THE MID-HILLS OF
CENTRAL HIMALAYA**

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The study addressed the integrated effect of seasons, slope aspect and land use on soil faunal population density, diversity and QBS-ar in the mid-hills of the central Himalaya, Nepal. One of the objectives was to determine the relationship among soil biological and physio-chemical Indicators. Some investigated physio-chemical variables were pH, moisture, temperature, texture, bulk density and organic carbon of the soil. Soil samples were collected from two different land use type: agriculture and forest in pre-monsoon (April) and post monsoon (October) in 2009 in the middle position of both north facing and south facing slopes. Soil core sampling (10*10*10) was performed, and fauna was extracted by using the modified Berlese Tullgren funnel. Particle size analysis was carried out using the soil hydrometer method, soil pH was measured using a pH probe with glass-calomel electrode and 1:1 soil: water ratio, soil organic matter and Soil organic carbon was measured by Dry combustion method, soil temperature using a digital thermometer, soil moisture was determined by gravimetric method and bulk density was determined using the core method. Significant differences in soil biological and physio-

chemical indicators with respect to seasons, land use and slope aspect were determined using three way factorial ANOVA. Pearson's correlation was done to determine the relationship among the soil physio-chemical and biological indicators. Most of the physio-chemical properties differed significantly with respect to the land use, slope aspect and seasons. Soil faunal population density was highly statistically significant with the seasons ($p=0.000$) and land use ($p=0.009$), but non-significant with the slope aspect. Average faunal population density was higher in post monsoon (11245 individuals /m²) as compared to pre-monsoon (3765 ind./m²). Average faunal population density on both seasons was higher on north slope (pre monsoon: 4400 ind./ m²; post monsoon: 12660 ind./m²) than southern slope(pre monsoon: 3130 ind./m²; post monsoon: 9830 ind./m²). Faunal density was higher in the forest as compared to agricultural land in both seasons. Shannon Diversity Index was weakly significant ($p=0.05$) with the seasons while QBS-ar was highly significant different ($p=0.000$) with the seasons. Pearson's correlation indicated that soil moisture was highly correlated ($p=0.01$) positively with SOC and population density while negatively with soil temperature ($p=0.010$ in pre-monsoon. In post monsoon Bulk density was negatively correlated ($p=0.05$) with QBS-ar and population density ($p=0.05$). Results indicated that season, slope aspect and land use all had significant effects on soil biological and physio-chemical indicators. Soil moisture, temperature, soil organic carbon and bulk density appeared to be good indicators of soil quality and these factors also determine the density and diversity of soil fauna.

**SEX AND AGE CATEGORIES OF RODENTS IN THE PREY OF BARN OWL
(*TYTO ALBA*) COLLECTED FROM SOME LOCALITIES OF DISTRICT
THATTA, SINDH.**

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Barn Owls have been proposed as a means of rodent control in agricultural areas because they hunt rodents that damage crops. However, research on this topic has been inconclusive. Because of habitat alteration and abundant food, rodents are, extremely numerous in agricultural landscapes. Rodents can inflict considerable damage to crops and farm equipment resulting in financial losses for growers. Out of 421 regurgitated pellets of barn owl collected from six localities of District Thatta, Sindh, Pakistan. 169 (54.3%) skulls were of male rats/ mice and 142 (45.7%) were identified as female rats skulls. Among them 52 (13.2%) skulls were of Sub. Adult rats, 224 (56.7%) skulls of Adults rats and 119 (30.1 %) skulls of Older rats were found. The most dominant (54.3%) sex categories eaten by the owl were of male and the most dominant (56.7%) frequency were of Adult rats/ mice in the age categories eaten by the owl.

PREY SPECIES IN THE DIET OF THE BARN OWL (*TYTO ALBA*) AT SIX LOCALITIES OF DISTRICT THATTA, SINDH

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The family Tytonidae includes the Barn and Grass Owls; there are 11 species in this family. The members of this family are world wide in distribution. The Barn Owl *Tyto alba* (Scopoli, 1769) belongs to the Family Tytonidae, Order Strigiformes. Barn Owl are easily recognized by their large rounded skull with forward directed eye sockets, gives it a completely different appearance from other bird families (Robert, 1991). Field studies were conducted to determine the sites of the Barn Owl (*Tyto alba* Scopoli, 1769) in M.K. Soomro Goth (D2_{L1}), Ghulamullah (D2_{L2}), Pirpatho (D2_{L3}), Jatti (D2_{L4}), Warshah (D2_{L5}) and Bello (D2_{L6}), District Thatta, Sindh. Feeding potential of the barn owl as a predator has been evaluated. During present research studies 20 regurgitated pellets which were collected from M.K. Soomro Goth (D2_{L1}), 79 pellets collected from Ghulamullah (D2_{L2}), 161 pellets collected from Pirpatho (D2_{L3}), 33 pellets collected from Jatti (D2_{L4}), 61 pellets collected from Warshah (D2_{L5}) and 67 pellets collected from Bello (D2_{L6}), District Thatta, Sindh, were examined/ analyzed in the laboratory with the help of reference material, literature and identification keys. Out of total 421 regurgitated pellets examined, bony remains of *Millardia meltda* were 98 (18.9%), *Bandicota bengalensis* were 54 (1.4%), unidentified rat spp. were 35 (6.8%), *Rattus rattus* were 20 (3.9%), *Mus musculus* were 78 (15.1%), *Nesokia indica* were 28 (5.4%), *Tatera indica* were 71 (13.7%), *Suncus murinus* were 130 (25.1%), *Suncus stoliczkanus* were 2 (0.4%), unidentified shrews were 2 (0.4%), *Trophozous sp.(bat)* were 4 (0.7%), insect remains were 8 (10.8%), birds were 53 (9%), amphibians (Frogs) were 5 (6.8%) and plant material was 4 (5.4%).

PROSPECTS AND LIMITATIONS OF BUFFALO PRODUCTION IN PUNJAB IN PAKISTAN

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A total of three hundred (300) respondents were interviewed from the selected districts of various zones of Punjab during a survey, to study the prospects and limitations of buffalo production kept under different production systems. Buffalo production in

Punjab is being practiced under four different systems viz; rural subsistence (RS), rural market oriented (RMO), peri-urban (PU) and Commercial (Com) farming respectively. Only those respondents were interviewed from various districts located in different parts of Punjab province where buffalo production was the main source of income. The field data collected through personal face-to-face interviews by field teams were analyzed using computer software "SPSS". The results indicated that overall literacy rate among buffalo producers was 66.3 %. It was highest in the per-urban (PU) buffalo producers and lowest in the case of buffalo producers of rural subsistence (RS) production system. The majority of the buffalo owners were agriculturists whereas others belong to non-agricultural professions. The mean herd profile of an average buffalo producer was: young males below one year (8.1%), young females below one year (22.1%), mature males (3.6 %), mature females (23.0%), milch adults (29.9%), dry females (11%), breeding males (1.3%) and draught animals (1.7%). About 47.6 % farmers demanded for the provision of better infrastructure and improvement in marketing system, while 10.5 % suggested the need of specific livestock market. Whereas 37.5 % pointed out the need for provision of credit facilities and 6.2 % demanded the establishment of market with proper legislation. Various practices regarding feeds and fodder adopted by the buffalo farmers have been presented. It can be concluded that a lot of improvement is possible in buffalo production through the adoption of proper feeding and management practices under field conditions. Majority of the farmers were not aware of many technological interventions, which indicated the need of establishing an efficient livestock extension services.

SEX CATEGORIES OF RODENTS IN THE PREY OF BARN OWL (*TYTO ALBA*) COLLECTED FROM THREE LOCALITIES OF DISTRICT KARACHI, SINDH

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Barn Owl naturally nest and roost in barns, silos, haystacks in barns, graveyards, tree cavities, stream bank holes, palm trees, derelict houses, ruins, church towers, old chimneys, hollow trunk or large hollow branch and Agricultural buildings are the most commonly used. They feed almost small mammals. The barn owls are efficient predators and often take more of some species of prey than others, and may have important effect on numbers and community structure. Barn owl has consumed different sex components within populations of prey species. Out of 198 regurgitated pellets of barn owl collected from three localities; Port Qasim (L1), Baili (L2) and Malir Agricultural Farms (L3) of District Karachi, Sindh, Pakistan. 101 (57.4%) skulls were of male rats/mice and 75 (42.6%) were identified as female rats skulls. The most dominant (54.4%) sex categories eaten by the owl were of male

**NEW REMAINS OF *GAINDATHERIUM VIDALI* FROM UPPER CHINJI
FORMATION OF THE SIWALIKS, PAKISTAN**

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New remains of *Gaindatherium vidali* are described from the upper Chinji formation or the Lower Siwaliks. The specimens were collected from a newly discovered locality in Lava, District Chakwal. The sample comprises four specimens belonging to the upper dentition of the extinct rhinoceros, *Gaindatherium vidali*. Previously this species is known only in the Nagri Formation of the Middle Siwaliks of Pakistan. Present findings are a new addition to the known stratigraphic range of the species.

**MORPHOLOGICAL STUDY OF UPPER DENTITION OF TERTIARY
HIPPARION FROM DHOK PATHAN FORMATION**

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Hipparion specimens were collected, described and figured in this paper. The specimens have been collected from the tertiary hills of the village Dhok Pathan of the Middle Siwalik subgroup and comprise three isolated upper premolar and molars. The genus is well known in the Tertiary of the Siwalik Hills of North India, Kashmir and West Pakistan. The cheek teeth are hypsodont and quadrate in appearance. The protocone is very elongated somewhat flattened but round to oval in shape, and hypoconal groove is prominent in the studied specimens, which is the characteristics of Siwalik *Hipparion*. On the basis of sharp comparisons and similarities with previously described specimen of *Hipparion* the present material is ascribed to the *Hipparion antilopinum*.

**TAXONOMIC STUDY OF LOWER DENTITION OF *HIPPARION* FROM
MIDDLE MIOCENE DHOK PATHAN FORMATION OF PAKISTAN**

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Hipparion fossils collected from Dhok Pathan Formation (Middle Miocene) are described and figured in this paper. The sample comprises one isolated lower premolar and two isolated lower molars of *Hipparion antilopinum*. One isolated lower molar and fragment of mandibular ramus are identified as *Hipparion theobaldi*. Based upon the characteristic features and comparative study the present collection is ascribed to *Hipparion antilopinum* and *Hipparion theobaldi*.

THE STATUS OF WILDLIFE AND WILDLIFE MANAGEMENT IN PAKISTAN

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Wildlife includes all free-ranging vertebrates in their naturally associated environments. Wildlife may also be considered to include all plants and animals in wild ecosystems. Wildlife managers are concerned with managing habitats, including vegetation and invertebrates that are foods or disease vectors for vertebrates. The objectives of wildlife management in Pakistan are however to favour or control the abundance or distribution of vertebrates, the emphasis being mainly on the game species until the near past. With increasing awareness about the environment management of species other than game is also being undertaken. Most wildlife management is directed towards birds and animals. Fisheries have developed separately as a science hence not included in wildlife management. Reptiles have recently joined the foray.

Pakistan has some quality wildlife: Ungulates like Marco Polo's sheep, Urial, Markhor, Himalayan ibex, Blue sheep, Wild goat, Grey goral, Blackbuck, Chinkara, Goitered gazelle, Nilgai; Musk deer, Hog deer, Barking deer; Carnivores like Snow leopard, Common leopard, Brown bear, Black bear, Indian wolf, various Cats e.g. Leopard cat, Caracal, Lynx, Foxes and the pests like wild boar and jackals. Indus River blind Dolphin is endemic to Pakistan. Marine mammals include the dolphins and porpoises and also some whales.

Among the birds there are pheasants like Western Tragopan, Himalayan Monal,

Koklas, White-crested Kaleej, Cheer, Indian Peafowl; partridges like Chakor, Ram Chakor, Grey and Black Francolins, Snow partridge, See-see partridge; Quails, Sand grouse, Coursers, Pigeons and Doves; a great variety of song birds; migratory Houbara bustard and waterfowl like Greylag goose, Bar-headed goose, Mallard, Pintail, Pochard, Widgeon, Garganey, Gadwall, Shoveler, Golden eye Northern teal, Tufted, Coot and a variety of waders; birds of prey like Golden eagle, Imperial eagle, Osprey, Shikra, Hobby, Kestrel, Lesser Kestrel, Merlin, falcons like Cherrug, Peregrine, Luggar and Vultures including Himalayan griffon, Lammergeyer, Black, Egyptian and White-backed.

Reptiles include the Marsh crocodile, Gavial, marine turtles like Green and Olive Ridley, fresh water turtles, soft-shelled turtles and land tortoises, venomous snakes e.g., Indian cobra, Vipers, Krait, Sea snakes, non-poisonous Colubers; different lizards and Agamas.

Amphibians of note are frogs, toads and salamanders.

Wildlife populations over the years have generally been declining, as apparent from IUCN Red Data Book listing; the causes being habitat destruction, unsustainable hunting, weak policies and law enforcement, lack of political will, unaware public, and above all lack of capacity both in terms of human resource and financial resource.

Wildlife management is the art of making land produce valuable populations of wildlife and consists of controlling the number, distribution, and quality of wild animals, either directly ---as by manipulating hunting seasons---or indirectly---as by manipulating wildlife habitat. The job is assigned to the Wildlife Departments in the provinces. The management is accorded through taking wildlife measurements, analyzing the data for extensive or intensive management and following the principles of management. All this is done against the odds---public pressures, constraints and lack of resources. Management also leads to Conservation in the present scenario. Wildlife conservation is a social process encompassing both lay and professional activities that define and seek to attain wise use of wildlife resources and maintain the productivities of wildlife habitats. This is an integrated process where the public and private partnership is considered necessary. Pakistan has just started this endeavour with the the help of local communities and NGOs like IUCN and WWF.

SECTION - VI**POSTER SESSION****INHIBITION OF CELLULOLYTIC ACTIVITY ISOLATED FROM *TRIBOLIUM CASTANEUM* BY LEAVE EXTRACTS OF *DATURA FASTUOSA***

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Cellulolytic activity isolated from common red Flour beetle (*Tribolium Castaneum*) was screened against leaves extracts of *Datura fastuosa*. Leaves of plants were dried and macerated in ethanol. The ethanolic extracts were further characterized by different tests TLC and FTIR. Inhibition of cellulolytic activity was studied by substrate agar plate assay and DNS method. Ethanolic extracts of *Datura fastuosa* inhibited the cellulase activity. Silica column chromatography Ethanol soluble extract was performed. Active fractions were subjected to TLC and FTIR. Tannin and flavonoids were found in the active pooled fractions. The study showed that ethanolic leaves extracts of *Datura fastuosa* caused complete inhibition of endoglucanase and exoglucanase activities.

HEAVY METALS AND ANTIBIOTIC RESISTANT COLIFORM BACTERIAL CONTAMINATION IN FRUIT CHAT SAMPLES FROM LAHORE

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Street vended ready to eat foods, especially those served semi and uncooked are implicated in the food borne bacterial infectious diseases. Unhygienic conditions during processing of such foods may lead to certain epidemics. In the present study five fruit chat samples collected from different areas of Lahore, were processed for detection and enumeration of coliforms. Thirty one strains of coliforms bacteria were isolated from the samples. Ninety percent, 83% and 80% of the isolates were found resistant to Vancomycin (VA 30), Oxacillin (Ox 10), and Amoxicillin (AML 25), respectively while 17%, 65 and 17% of the isolates were found resistant to Chloramphenicol (CA30), Nalidixic acid (Na 30) and Rifampicin (RD 30) respectively. Of the bacterial isolates

23% showed resistance to Cr and 20 % to Cu, whereas all the strains were found sensitive to Hg and Ag. The zones of growth inhibition against hg and Ag ranged from 24 to 42mm and 12 to 22 mill, respectively. These results showed faecal contamination of the street vended food. Moreover antibiotics and heavy metal resistance of the coliforms bacterial isolates indicated their anthropogenic and urban origin. It can easily be speculated that the food samples. might had been carrying enteric pathogens well resistant to the frequently prescribed antibiotics. Lack of hygienic measures and spread of enteric infections through such food is indicated.

COMPARATIVE PROFILE OF FREE IONS IN SPIDERS OF CITRUS

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Most common local occurring spiders were selected for the determination of free ions of venom and hemolymph. The comparison between free ions found in venom and body fluid of these species showed characteristically significant differences. High concentration of K^+ was found in venom as compared to the body fluid. K^+ and Ca^{2+} concentrations were lower in females as compared to their male counterparts. Only females of the genus *Myrmarachne* had a low concentration of K^+ and Na^+ while *Sparassidae* sp. had highest K^+ contents that were significantly different. There was a non significant difference in Ca^{2+} contents of venom and heamolymph. The differences and variations were mostly species specific.

INCREASE IN BODY WEIGHT DEPENDS ON DIETARY COMPOSITION AND PALATABILITY OF THE DIET IN RATS

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Non-alcoholic fatty liver disease (NAFLD) is characterized by the buildup of fat in the liver cells of people who do not drink alcohol excessively. It refers to a wide spectrum of liver damage, ranging from simple steatosis to NASH (non-alcoholic steatohepatitis), advanced fibrosis and cirrhosis. The pathogenesis of fatty liver is likely

to depend on a complex interaction of environmental and genetic factors. NAFLD is thought to be related mainly with insulin resistance (IR) syndrome and oxidative stress, metabolic syndrome, Obesity, etc. The aim of the current study is to develop abdominal obesity leading to fatty liver in Wistar rats using different formulations of fatty diet. Wistar rats of 200g were fed on different diets for eight weeks. The control group was given normal rat chow whereas the experimental group was given modified chow with additional fat component (33% fat). The animals were kept in 12h dark and light cycle under controlled temperature and humidity. Food consumption, Water intake, and increase in body weight of the control and experimental groups were recorded on daily basis for 7 weeks. The animals gained weight gradually until the end of 5th week however, after 5 weeks there was no further weight gain in the experimental group. The weight gain in the experimental group was significantly different from the control group right from the very first week, which can be attributed to the diet composition ($P=0.0020$). The food consumption and water intake by the animals of the control as well as experimental group declined with the course of time. This decline in food consumption was significant in the experimental group at 5th week of the study as compared to the 1st week ($P<0.05$) and for water intake this decline was significant at 4th week and highly significant at 6th week of study when analyzed by one way ANOVA. Taken together these results we conclude that the diet pattern significantly affect the animal weight as well as the feeding habits. Further studies on the impact of high fat diet on liver and body hematology are ongoing.

PREDICTION OF MODIFICATION POTENTIAL OF PHOSPHO-TYROSINE UTILIZING NEURAL NETWORKS WITH MAPRES RULE INDUCTION

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Prediction models for PTMs are generally developed using data driven learning methods. These methods tend to learn the induction of statistical correlation in the sequence data. Models evolved by the data learning usually results in large number of false positive and false negative prediction. This study reports a prediction model with a new approach to evolve prediction methods by learning sequence data with rule induction. This new approach was applied on phospho-tyrosines to develop a prediction model. In the study MAPRes was applied to phospho-tyrosine to mine association rules. These association rules were then used as induction input along with the sparse encoded amino acid sequence data.

**MAPRes1.1: PREFERENCE AND PROPERTY BASED ASSOCIATION RULE
MINING ALGORITHM FOR POST-TRANSLATIONAL MODIFICATION
DATA OF PROTEINS**

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After translation is completed proteins are targeted by number of biochemical processes - particularly post translational modification (PTM). The general rules related to protein interactions are not well established. Therefore, bioinformatics methods are required to define the capability of the protein to perform multiple functions in the environment where multiple proteins exist. Recently an algorithm MAPRes was designed to analyze PTMs. Indeed number of significant rules were discovered and reported by MAPRes. MAPRes does not provide support to perform association rule analysis on biophysical and/or biochemical properties and can only perform analysis of the protein sequence surrounding the PTM sites. This study reports a new version of MAPRes 1.1. It has data inconsistency and preprocessing (DIP) module that performs Sequence Consistency Analysis, Modified and Position Profile Analysis and Duplicate Entry Analysis. This new version has the capacity to analyze positive and negative examples which can be used for future prediction of certain biological events. Moreover it provides the mechanism to mine association rules utilizing the amino acid properties.